Appendix D. Individual Acute, 8-Hour, and Chronic Reference Exposure Level Summaries

D.1 Summaries using this version of the Hot Spots Risk Assessment guidelines

D.2 Acute RELs and toxicity summaries using the previous version of the Hot Spots Risk Assessment guidelines (OEHHA 1999)

D.3 Chronic RELs and toxicity summaries using the previous version of the Hot Spots Risk Assessment guidelines (OEHHA 2000)

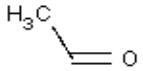
Appendix D.1 Summaries using this version of the Hot Spots Risk Assessment guidelines

Substance		Inhalation REL ($\mu g/m^3$)	Oral REL ($\mu g/kg$ BW-day)	Hazard Index Target Organs	Human Data
Acetaldehyde (75-07-0)	**A**	470		Sensory irritation; bronchi, eyes, nose, throat	X
	8	300		Respiratory system	
	C	140		Respiratory system	
Acrolein (107-02-8)	**A**	2.5		Sensory irritation; eyes	X
	8	0.7		Respiratory system	
	C	0.35		Respiratory system	
Arsenic (7440-38-2) **& inorganic arsenic compounds (including arsine)**	**A**	0.20		Development (teratogenicity); cardiovascular system; nervous system	
	8	0.015		Development; cardiovascular system; nervous system; lung; skin	X
	C	0.015	0.0035	Development; cardiovascular system; nervous system; lung; skin	X
Formaldehyde (50-00-0)	**A**	55		Sensory irritation; eyes	X
	8	9		Respiratory system	X
	C	9		Respiratory system	X
Manganese (7439-96-5) **& manganese compounds**	**A**	--			
	8	0.17		Nervous system	X
	C	0.09		Nervous system	X
Mercury (7439-97-6) **& inorganic mercury compounds**	**A**	0.6		Nervous system, development	
	8	0.06		Nervous system	X
	C	0.03	0.16	Nervous system	X

Acetaldehyde Reference Exposure Levels

(ethanal; acetic aldehyde; acetylaldehyde; ethylaldehyde; diethylacetyl)

CAS: 75-07-0



1. Summary

Based on acute and chronic inhalation studies conducted mostly in experimental animals, the target tissue for acetaldehyde has consistently been at the portal of entry with effects occurring primarily in the upper respiratory tract at lowest concentrations. The major noncancer health effects of acute exposure in humans to acetaldehyde vapors consist of irritation to the eyes, skin, and respiratory tract. Low to moderate air concentrations (25 ppm to 200 ppm) cause eye and upper respiratory tract irritation. Moderate concentrations (~ 300 ppm or greater) also cause bronchoconstriction in asthmatics as measured by a greater than 20% drop in forced expiratory volume (FEV_1). Signs of acute toxicity in animals at high concentrations (~10,000 ppm) include labored respiration, mouth breathing, weight loss, and liver damage. The studies described in this document include those published through the Spring of 2008.

OEHHA used the critical effect of bronchoconstriction in asthmatics as the basis for determination of the acute Reference Exposure Level (REL).

Subchronic and chronic exposure to acetaldehyde causes inflammation and injury to the respiratory tract (e.g. lesions including hyperplasia and metaplasia of the olfactory mucosa). Exposure to acetaldehyde, as seen in experimental animal studies, causes histopathological changes in the nose, larynx, and trachea including degeneration, hyperplasia, and metaplasia. Chronic toxicity to rats and hamsters following inhalation exposure to acetaldehyde includes increased mortality and growth retardation. OEHHA used degenerative, inflammatory and hyperplastic changes of the nasal mucosa in rats as the basis for the 8-hour and chronic REL.

Children, especially those with diagnosed asthma, may be more likely to show impaired pulmonary function and symptoms of asthma than are adults following exposure to acetaldehyde. Acetaldehyde is identified as a Toxic Air Contaminant (TAC); this report presents evidence that it should also be listed as having the potential to differentially impact infants and children due to its effects as a respiratory irritant and possible exacerbation of asthma. In addition, acetaldehyde has high California Hot Spots and mobile source emissions, and secondary formation in the atmosphere (OEHHA, 2001).

1.1 Acetaldehyde Acute REL

Reference Exposure Level	**470 μg/m^3 (260 ppb)**
Critical effect(s)	Sensory irritation, broncoconstriction, eye redness and swelling
Hazard index target(s)	Bronchi, eyes, nose, throat

1.2 Acetaldehyde 8-Hour REL

Reference Exposure Level	**300 μg/m^3 (160 ppb)**
Critical effect(s)	Degeneration of olfactory nasal epithelium
Hazard index target(s)	Respiratory system

1.3 Acetaldehyde Chronic REL

Reference Exposure Level	140 g/m3 (80 ppb)
Critical effect(s)	Degenerative, inflammatory and hyperplastic changes of the nasal mucosa in animals
Hazard index target(s)	Respiratory system

2. Physical & Chemical Properties

Description	Colorless liquid or gas (above 21°C)
Molecular formula	C_2H_4O
Molecular weight	44.05 g/mol
Density	0.79 g/cm^3
Boiling point	21 °C
Melting point	-123.5 °C
Vapor pressure	755 mm Hg @ 20°C
Odor threshold	0.09 mg/m^3
Solubility	Miscible in all proportions with water and the most common organic solvents.
Conversion factor	1.8 mg/m^3 = 1 ppm @ 25°C

3. Occurrence and Major Uses

Acetaldehyde is used as an intermediate for the manufacture of a number of other chemicals, including acetic acid, acetic anhydride, ethyl acetate, peracetic acid, pentaerythritol, chloral, alkylamines, and pyridines (HSDB, 2004). Sources of acetaldehyde emissions include interior finish materials such as sheet vinyl flooring and carpets, and wood-based building products such

as fiberboard and particleboard. Some consumer products also emit acetaldehyde, including adhesives and glues, coatings, lubricants, inks, nail polish removers, liquid wax for wood preservation, detergent and cleansers, deodorants, fuels, and mold inhibitors (Beall and Ulsamer, 1981; CARB, 1993). Emissions of acetaldehyde also occur during combustion processes such as cigarette smoking, automobile exhaust, and use of fireplaces and woodstoves, although long-term indoor concentrations tend to be dominated by non-combustion sources.

An emissions study of new building materials found that samples of carpet, fiberboard, particleboard, and non-rubber resilient flooring emitted acetaldehyde (Burt et al., 1996; IWMB, 2003). Air concentrations based on the acetaldehyde emission rates from these various building products, when modeled to standard State office and classroom dimensions, ranged from 4.6 to 26 $\mu g/m^3$ (2.6 to 14 ppb).

Indoor concentrations of acetaldehyde often greatly exceed outdoor levels and appear to dictate personal exposures, which is consistent with the more significant and widespread indoor sources of this aldehyde. In 2002, the annual average outdoor concentration of acetaldehyde in the South Coast Air Basin was 2.5 $\mu g/m^3$ (1.4 ppb). In Brazil, which has a high usage of ethanol as a transportation fuel, outdoor acetaldehyde concentrations have been measured as high as 63 $\mu g/m^3$ (35 ppb) while a highway tunnel had measured levels of acetaldehyde of 430 $\mu g/m^3$ (240 ppb). The mean acetaldehyde concentrations in U.S. homes range from 15 to 36 $\mu g/m^3$ (8.3 to 20 ppb), but reached as high as 103 $\mu g/m^3$ (57.2 ppb) in newly manufactured homes (Zweidinger et al., 1990; Lindstrom et al., 1995; Hodgson et al., 2002; Kinney et al., 2002). Acetaldehyde concentrations measured in Southern California portable classrooms ranged from 5.7 to 12.8 $\mu g/m^3$ (3.2 to 7.1 ppb) with a mean of 9.8 $\mu g/m^3$ (5.4 ppb) (Hodgson et al., 2004) Similar concentrations were found in classrooms of the main buildings. Measured concentrations of acetaldehyde in public/office buildings range from 3 to 16 $\mu g/m^3$ (1.7 to 8.9 ppb).

Environmental tobacco smoke (ETS) has been found to be a source of environmental acetaldehyde. Although long-term acetaldehyde levels in smoking and non-smoking homes tend to be similar, acetaldehyde concentrations in homes as a result of exposure from ETS for nonsmoking Californians has been estimated at 11-15 $\mu g/m^3$ (6.1 to 8.3 ppb) (Miller et al., 1998). Concentrations of acetaldehyde measured over a 72-hour period in 57 homes ranged from 3 to 23 $\mu g/m^3$ (1.7 to 12.8 ppb). However, no significant difference was observed between the homes of smokers and nonsmokers (Brown et al., 1994). A 48-hour integrated measurement of breathing-zone concentrations revealed that people who work in garages (9 smokers and 13 nonsmokers) had significantly higher levels of breath acetaldehyde than controls (4 smokers and 11 nonsmokers), and the smokers had significantly higher levels of breath acetaldehyde than the nonsmokers.

The concentration of breath acetaldehyde (endogenous level) in non-alcoholic, non-smokers range from 0.7 to 11.0 $\mu g/m^3$(0.4 to 6.1 ppb), but can be somewhat higher in smokers (16 ± 3 $\mu g/m^3$ = 8.9ppb). The higher concentrations are seen in the breath of smokers after they ingest alcohol. With alcohol consumption, the concentrations of acetaldehyde produced vastly exceed the trace amounts generated from microorganisms or other possible endogenous substrates. When subjects with normal aldehyde dehydrogenase (ALDH) activity drink small amounts of alcohol (0.4-0.8 g/kg), the concentrations of breath acetaldehyde may reach between 200 and 2200 $\mu g/m^3$ (111 to 1222 ppb) (Shaskan and Dolinsky, 1985; Jones, 1995).

In a controlled human study, five healthy nonsmoking adults inhaled low doses of ethanol (ETOH) and concentrations of ETOH and acetaldehyde were measured in the alveolar air using only the last portion of air in the sampling bag after forced expiration through a three-way valve (Tardif et al., 2004). Exposures were for six consecutive hours to 25, 100, or 1000 ppm ETOH. After two hours of exposure at 25 ppm, acetaldehyde and ETOH were measured in the alveolar air at 0.06 and 7.5 ppm, respectively.

In Asian subjects with a genetic deficiency of the enzyme aldehyde dehydrogenase (ALDH), the concentration of acetaldehyde in the breath after drinking can reach 8.8-22 mg/m^3 (4.9 to 12.2 ppm). Higher concentrations of acetaldehyde have been shown to activate mast cells, which then induce histamine release. In one case study, a patient had a severe bronchial asthma attack after ingesting food containing small amounts of alcohol, and was found to be homozygous for the ALDH-2 mutant genotype. Both acetaldehyde and ethanol inhalation tests were performed on the patient. The ethanol inhalation test was negative, but acetaldehyde inhalation (5, 10, 20, or 40 mg/ml) decreased $FEV_{1.0}$ by 33.5% at 20 mg/ml (Saito et al., 2001).

4. Disposition

Acetaldehyde is readily absorbed through the lungs into the blood following inhalation exposure. Acetaldehyde is rapidly exchanged and equilibrated between blood entering the lungs and alveolar air. Male Sprague-Dawley rats exposed to acetaldehyde vapor concentrations in air ranging from 9 to 1000 mg/l (0.009 to 1 mg/m^3 or 500 to 555 ppb) for one hour had higher levels of acetaldehyde in the blood than liver (Watanabe et al., 1986). Levels in the arterial blood were also higher than in peripheral venous blood.

Two studies were performed using humans and dogs to determine the percent retention of inhaled acetaldehyde in the respiratory tract (Egle Jr, 1970; Egle Jr., 1972a; 1972b). In humans, the total respiratory tract retention of acetaldehyde was 45-70% when inhaled either orally or nasally (Egle Jr, 1970). Physiological respiratory total retention in multiple breath experiments was independent of tidal volume, and uptake was controlled by frequency and duration of ventilation. Total respiratory tract retention of acetaldehyde in dogs was found to be very close to human retention values and inversely related to ventilatory rate in the same manner as humans (Egle Jr., 1972b). Uptake was also found to be higher in the upper than the lower respiratory tract and unrelated to changes in concentration inhaled or tidal volume (Egle Jr., 1972b).

Acetaldehyde deposition efficiency is strongly dependent on the inspired concentration, with deposition being less efficient at high compared to low concentrations. Species differences have been observed in uptake efficiency with uptake being significantly higher in the mouse, rat, and hamster compared to the guinea pig at 100 ppm, but at 10 ppm the rat had the lowest uptake (Morris, 1997a).

Following oral administration, acetaldehyde is readily absorbed from the gastrointestinal tract and undergoes extensive first pass metabolism in the liver; only 5% remains unchanged (Morris, 1997b).

Acetaldehyde rapidly diffuses through cellular membranes and is distributed to various organs for metabolism. The half-life in rats after inhalation of actetaldehyde was 10 minutes, and the

time to total body clearance was 40 minutes (Shiohara et al., 1984). Inhaled acetaldehyde does not undergo a first pass effect and is distributed to all tissues including the liver. Inhaled acetaldehyde undergoes extrahepatic metabolism and is metabolized by aldehyde dehydrogenase in the lungs to acetate. Aldehyde dehydrogenase is found in both the cytosol and the mitochondria. Inhaled acetaldehyde undergoes extrahepatic metabolism by the respiratory-olfactory epithelium, kidneys, blood, brain, and spleen, and only small amounts reach the liver. Acetaldehyde also crosses the blood-brain barrier. Protons (H^+) are a by-product of acetaldehyde metabolism (to acetate), which under high exposure conditions, have the potential to acidify cells and cause cytotoxicity, if cellular buffering systems and proton pumps are overwhelmed (Bogdanffy et al., 2001).

Various isoenzymes of alcohol dehydrogenase transform ethanol into acetaldehyde, which in turn is rapidly oxidized by aldehyde dehydrogenase (ALDH) into acetate. Both pathways for acetaldehyde metabolism (low-affinity (cytosolic ALDH1) and high-affinity (mitochondrial ALDH2) are present and have been described in rodent nasal olfactory and respiratory tissues (Casanova-Schmitz et al., 1984; Morris, 1997a; 1997b; Bogdanffy et al., 1998).

Functional genetic polymorphisms and ethnic variation exist at various genes encoding these enzyme proteins which all act to alter the rate of synthesis of the toxic metabolite acetaldehyde, or decrease its further oxidation. About 50% of the Asian population are alcohol-sensitive, having a deficiency or low activity in aldehyde dehydrogenase enzymes that are important in ethanol metabolism. This can result in high acetaldehyde levels in blood and breath following alcohol consumption.

A small amount of acetaldehyde is produced in the body during normal intermediary metabolism and is also a product of microbial fermentation of sugars in the gut. However, based on studies in animals, the critical effects of exposure to exogenous acetaldehyde occur at the site of initial contact (i.e., the respiratory tract following inhalation).

At least two isozymes of aldehyde dehydrogenase were found in the rodent nasal mucosa, differing with respect to their apparent Vmax and Km values (Morris, 1997a). Male F344 rats were exposed to 1500 ppm acetaldehyde for 6 hours/day for 5 days. Oxidation of acetaldehyde occurred more rapidly in the homogenates of the respiratory than the olfactory mucosa (Morris, 1997a). The nasal tissue is the first to contact acetaldehyde vapors upon inhalation. The aldehyde dehydrogenase acts as a defense mechanism helping to minimize or prevent toxic injury to nasal tissues exposed to airborne compounds. Pretreatment with an ALDH inhibitor reduced nasal acetaldehyde deposition rates (Morris, 1997a).

Acetaldehyde can be eliminated unchanged in urine, expired air, and skin (Baselt and Cravey, 1989) and is metabolized by aldehyde dehydrogenase to acetate which is readily excreted in the urine.. Acetaldehyde is highly reactive and can bind to amino acids and blood and membrane proteins, and act as a hapten (Mohammad et al., 1949; Eriksson et al., 1977; Gaines et al., 1977; Donohue Jr. et al., 1983; Tuma and Sorrell, 1985; Dellarco, 1988; Hoffmann et al., 1993; Wickramasinghe et al., 1994; Tyulina et al., 2006). Antibodies against acetaldehyde conjugates have been detected in human and rabbit serum (Gaines et al., 1977). Acetaldehyde is a weak clastogen that induces sister chromatid exchanges and reacts with DNA to form DNA-protein

and DNA-DNA cross-links (Dellarco, 1988). Acetaldehyde causes lipid peroxidation, which can lead to adduct formation and free radical-induced cell injury.

5. Acute Toxicity of Acetaldehyde

5.1 Acute Toxicity to Adult Humans

Several studies in human volunteers are available, including several recent studies in asthmatics where subjects inhaled aerosolized acetaldehyde. The ability to determine a one-hour reference exposure level (REL) is limited due to the extremely short exposure period of only 2-4 minutes that was used in these studies. However, inhalation experiments with human volunteers in which exposure lasted longer are old and of limited design. The major acute effects of human exposure to acetaldehyde vapors consist of irritation to the eyes, skin and respiratory tract, and bronchoconstriction in asthmatics. The key study used to determine the acute Reference Exposure Level (REL) was a study performed in human volunteers investigating bronchoconstriction in response to inhaled aerosolized acetaldehyde (Prieto et al., 2000). The Prieto et al. (2000) study determined the mean acetaldehyde concentration causing a 20% decrease in Force Expiratory Volume (FEV_1) in asthmatic human volunteers.

Silverman et al. (1946) exposed human volunteers to acetaldehyde to determine the sensory response limit for solvent concentrations when estimating ventilation requirements for comfortable working conditions (Silverman et al., 1946). The sensory limits were reported and compared to the maximum allowable concentration, which was stated as 200 ppm for acetaldehyde at the time of the study. Twelve volunteer human subjects of both sexes were used for each solvent exposure. During the 15 minute exposure period, motion pictures were shown to occupy the subjects' attention and divert their thoughts from the atmospheric exposure in the chamber. The results, though described in a limited way, are useful because the analysis was performed in human subjects and the concentrations tested were as low as 25 ppm. At 50 ppm, the majority of subjects experienced eye irritation (number not specified). The subjects that did not report eye irritation had reddened eyelids and bloodshot eyes after exposure at 200 ppm. Several subjects reported unspecified irritation at 25 ppm and "objected strenuously." Finally, nose and throat irritation were reported as occurring at concentrations greater than 200 ppm (Silverman et al., 1946).

A second acute human study was found in the historical literature, where fourteen male subjects were exposed to 134 ppm acetaldehyde in a chamber for 30 minutes (Sim and Pattle, 1957). Subjects reported mild upper respiratory tract irritation (Sim and Pattle, 1957). However, a major confounder with this study appears in the methods section, which stated that subjects were permitted to smoke inside the "chamber" during the 30 minutes.

Acetaldehyde provocation tests have been conducted with asthmatic and non-asthmatic human subjects using aerosolized acetaldehyde solutions. As mentioned previously, aldehyde dehydrogenase plays an important part in the metabolism of ethanol in making possible the conversion of acetaldehyde (previously formed from ethanol by alcohol dehydrogenase) to acetic acid. Lower activity of aldehyde dehydrogenase leads to elevated concentrations of acetaldehyde in the blood, which in asthmatic subjects may produce bronchoconstriction. There are indications that enhanced release of histamine from pre-activated airway mast cells plays an

important role (Myou et al., 1993). As a result of the polymorphism of ALDH-2, nearly half of the Japanese patients with asthma show bronchoconstriction after drinking alcohol, a phenomenon that is also known to occur in other Asian populations (Myou et al., 1993; Myou et al., 1994; Fujimura et al., 1999). In several studies in asthmatic volunteers, inhaled acetaldehyde aerosol has been tested for its bronchoconstrictive effect, first in three studies in Japanese subjects (Myou et al., 1993; Myou et al., 1994; Fujimura et al., 1999) and subsequently in several studies in Caucasian subjects (Prieto et al., 2000; Prieto et al., 2002a; Prieto et al., 2002b). In these studies, subjects inhaled aerosolized acetaldehyde for very short periods; exposure was (2-4 minutes).

Myou et al. (1993) exposed a group of nine asthmatic volunteers (age 39.2 ± 5.4 yr) and nine age- and sex- matched controls to aerosolized acetaldehyde for 2 minutes immediately followed by measurement of Force Expiratory Volume in one second (FEV_1). The solutions of 5, 10, 20, or 40 mg/ml of acetaldehyde were in saline and were inhaled from a nebulizer for 2 minutes by mouth tidal breathing wearing a noseclip. The aerosol was produced using a DeVilbiss 646 nebulizer operated by compressed air at 5 liters per minute. Nebulizer output was not reported but probably was the same as in later studies by this group, i.e. 0.14 ml/minute. No measurements of acetaldehyde concentration in air were made. The dose response study showed significant reductions in FEV_1 at all acetaldehyde test concentrations in asthmatics whereas no effect was seen in normal subjects (Myou et al., 1993).

In further experiments with the same group of volunteers, the influence of oral terfenadine, a histamine H1 blocker, was examined as was the bronchial responsiveness to methacholine (challenge with methacholine is a common asthma identification test). The response seen after inhalation of acetaldehyde was completely suppressed by pretreatment with terfenadine, which supports the hypothesis that bronchial hyper-responsiveness is a precondition of acetaldehyde induced bronchoconstriction, which is caused indirectly via histamine release in asthmatics (Myou et al., 1993). A rough estimate from the dose response curve as presented in the paper, suggests a PC_{20} for acetaldehyde (acetaldehyde concentration producing a 20% reduction in FEV_1) of about 20 mg/ml (Myou et al., 1993). The acetaldehyde aerosol concentration as mg/m^3 in this study can be estimated as follows. The nebulizer was operated at 5 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.14 ml/minute. When given at this rate a 20 mg acetaldehyde/ml solution (the estimated PC_{20}) corresponds to a concentration in air of approximately 560 mg/m^3 (about 314 ppm).

In a subsequent acute human study, nine asthmatic subjects of Japanese origin were used to determine whether bronchial responsiveness to inhaled methacholine (STET: a standard test used to identify agents that potentially exacerbate asthma) was altered when asthmatic subjects inhaled a sub threshold concentration of aerosolized acetaldehyde which did not cause bronchoconstriction, and whether any increase in bronchial hyper-responsiveness after acetaldehyde was mediated by histamine release (Myou et al., 1994). For each subject, the concentration of acetaldehyde producing a 20% fall in FEV_1 was determined (PC_{20}) using ascending doses (5, 10, 20, or 40 mg/ml) of acetaldehyde. The mean concentration of PC_{20} for the nine subjects was 23.3 mg/ml of acetaldehyde (Myou et al., 1994). The nebulizer was operated at 5 liters air/minute for 4 minutes with an acetaldehyde solution output of 0.14 ml/minute. Therefore, a 23.3 mg acetaldehyde/ml solution corresponds to a concentration in air of approximately 652 mg/m^3 (about 362 ppm).

In part two of this study, nine subjects inhaled a sub threshold concentration of 0.8 mg/ml acetaldehyde at 0.14 ml/minute for four minutes or saline followed by provocation with a range of increasing methacholine concentrations (Myou et al., 1994). FEV_1 was measured before and after treatment. Acetaldehyde potentiated bronchial hyper-responsiveness to provocation by methacholine (Myou et al., 1994) producing a marked reduction in PC_{20}-MCH (0.48 mg/ml versus 0.85 mg/ml after saline treatment) (Myou et al., 1994).

Myou et al. (1995) examined tachyphylaxis occurring in response to repeated inhalation of histamine or acetaldehyde in nine asthmatic subjects. The mean acetaldehyde concentration causing a 20% decrease in FEV_1 increased significantly from a geometric mean of 18.4 mg/ml (with a geometic standard error (GSEM) of 0.14) to 45.2 mg/ml (GSEM 0.14) over a period of one hour ($p<0.002$). The mean histamine concentrations causing a 20% decrease in FEV_1 were no different.

In a later study in asthmatics of Japanese origin, the hypothesis was tested that asthmatics that are sensitive to alcohol (showing bronchoconstriction after drinking alcohol) also have increased airway responsiveness to inhaled acetaldehyde when compared to asthmatics not sensitive to alcohol (Fujimura et al., 1999). Ten alcohol-sensitive asthmatics and 16 alcohol insensitive asthmatics (20-65 years) of Japanese origin inhaled acetaldehyde aerosol for 2 minutes by tidal mouth breathing and FEV_1 was measured. Increasing concentrations of acetaldehyde solutions in saline (0.04 to 80 mg acetaldehyde/ml) were inhaled until FEV_1 showed a fall of 20%. In the alcohol-sensitive group the geometric mean PC_{20} was 21.0 mg/ml (range not reported), whereas in the alcohol-insensitive group this was 31.7 mg/ml (range not reported). The difference between the groups, however, was not statistically significant (Fujimura et al., 1999). The aerosol was produced using a DeVilbiss 646 nebulizer operated by compressed air at 5 liters/minute with a nebulizer output of 0.14 ml/minute. The nebulizer was operated at 5 liters air/minute for 2 minutes with a acetaldehyde solution output of 0.14 ml/minute. At this rate, inhalation of acetaldehyde solutions of 0.04 to 80 mg/ml corresponds to concentrations in air of approximately 1.12 to 2240 mg/m^3. Similarly, the geometric mean PC_{20} in the alcohol-sensitive group corresponds to approximately 588 mg/m^3 (about 330 ppm) and the geometric mean PC_{20} in the alcohol-insensitive group to approximately 888 mg/m^3 (about 500 ppm).

In the key study (Prieto et al., 2000) used for the acute REL determination, the responses to methacholine and acetaldehyde challenges were measured in 81 non-smoking adults to determine differences in airway responsiveness between asthmatics and healthy subjects and to examine the relationship between acetaldehyde responsiveness and the variability of peak expiratory flow (PEF). Prieto et al. (2000) examined whether the bronchoconstriction seen in Japanese asthmatics after inhalation of acetaldehyde also occurred in Caucasian subjects. They exposed 61 mildly asthmatic subjects and 20 healthy subjects (control group) to aerosolized acetaldehyde (5 to 40 mg acetaldehyde/ml) for two minutes using a two-minute tidal breathing-method and FEV_1 was measured 60 to 90 seconds after inhalation of each concentration until FEV_1 dropped by more than 20%. In this study, the PC_{20} values for acetaldehyde ranged from 1.96 to 40 mg/mL with a geometric mean value of 17.55 mg/mL and the 95% confidence interval of the geometric mean (95% CI 4.72-38.3 mg/ml). Therefore, the lower limit of the geometric 95% CI was 4.72 mg/ml (Prieto, 2008). Effectively, the data are for the geometric mean (antilog of the mean PC20 acetaldehide) and the 95% CI is for the antilog of this value also. This is the traditional way these type of data are presented for acetaldehyde, methacholine or AMP (Prieto

2008). In the asthma group 56/61 subjects showed bronchoconstriction compared to 0/20 in the control group. Inhaled acetaldehyde was much less potent as a bronchoconstrictor than methacholine in asthmatic patients. Peak expiratory flow variation was significantly but weakly related to acetaldehyde responsiveness ($p = 0.004$). The results obtained by Prieto et al. (2000) indicate that airway hyper-responsiveness to acetaldehyde is a sensitive and specific indicator for separating normal and asthmatic subjects.

In the Prieto et al. (2000) study, aqueous solutions containing acetaldehyde were nebulized in a Hudson 1720 nebulizer operated by compressed air at 6 liters/minute with a nebulizer output of 0.18 ml/minute. Flow rates were reported in a National Advisory Committee document from the U.S. EPA, based on a personal communication from the Prieto group (NAS, 2004). The nebulizer was operated at 6 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.18 ml/minute. At this rate, inhalation of acetaldehyde solutions of 5 to 40 mg/ml corresponds to concentrations in air of approximately 150 to 1200 mg/m^3. The observed geometric mean PC_{20} of 17.55 mg/ml corresponds to 527 mg/m^3 (about 293 ppm) and the lower 95% confidence interval of 4.72 mg/ml corresponds to approximately 142 mg/m^3 (about 79 ppm).

In a follow-up study, Prieto et al. (2002a) exposed mildly asthmatic subjects (age 18-58 years) to 2.5 to 80 mg acetaldehyde/ml using a Hudson 1720 nebulizer with an output of 5 liters/minute. In the first group, 16 subjects were measured for their response to acetaldehyde which was compared to that of methacholine and adenosine-5'-monophosphate (two bronchoconstrictive agents of known potency). In the second group of 14 subjects, repeatability and side effects of acetaldehyde inhalation were examined. For acetaldehyde the PC_{20} ranged from 8.4 to 80 mg/ml with a geometric mean of 38.9 mg/ml (geometric mean values for methacholine and AMP were 0.6 and 17.4 mg/ml, respectively). The response to acetaldehyde was found to be moderately repeatable. For the group in which repeatability was examined, for acetaldehyde concentrations producing a >20% fall in FEV_1, most subjects had cough (64%), dyspnea (57%) or throat irritation (43%) (Prieto et al., 2002a). The nebulizer was operated at 5 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.16 ml/minute. At this rate, inhalation of acetaldehyde solutions of 2.5 to 80 mg/ml corresponds to concentrations in air of approximately 80 to 2560 mg/m^3. The observed geometric mean PC_{20} of 38.9 mg/ml corresponds to approximately 1245 mg/m^3 (about 692 ppm).

In a further volunteer study, Prieto et al. (2002b) studied comparative airway responsiveness to acetaldehyde (2.5 mg to 80 mg/ml) in subjects with allergic rhinitis (n=43), asthmatics (n=16), and healthy subjects (n=19). The number of subjects with a fall in FEV_1 >20% was 8/43 in the group with allergic rhinitis, 13/16 in the asthmatic group and 0/19 in the healthy subjects group. PC_{20} values in the group with allergic rhinitis ranged from 15.5 to 80.0 mg/ml with a geometric mean of 67.7 mg/ml whereas in the asthmatic group PC_{20} ranged from 8.4 to 80.0 mg/ml with a geometric mean of 35.5 mg/ml ($p < 0.001$) (Prieto et al., 2002b). The PC_{20} values in the allergic rhinitis group were also significantly lower than in the healthy control group ($p = 0.04$) (Prieto et al., 2002b). The nebulizer was operated at 5 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.16 ml/minute. Thus, inhalation of acetaldehyde solutions (2.5 to 80 mg/ml) corresponds to concentrations in air of 80 to 2560 mg/m^3. The observed geometric mean of 67.7 mg/ml corresponds to approximately 2166 mg/m^3 (about 1210 ppm) and the geometric mean of 35.5 mg/ml to approximately 1136 mg/m^3 (about 631 ppm).

As indicated above, the provocation tests involved acetaldehyde solutions that were aerosolized, and then inhaled by mouth. Aerosolized acetaldehyde solutions have been shown to be about 265 times less potent than methacholine in constricting the airways of asthmatic subjects, with aerosolized acetaldehyde solutions of 80 mg/ml resulting in cough, dyspnea, and throat irritation in the asthmatic subjects (Myou et al., 1993). In addition, the exposure times were very short (several minutes) and the concentrations eliciting a response in FEV_1 were much higher. However, it is important to note that in the Myou et al. (1994) study, aerosolized acetaldehyde potentiated bronchial hyper-responsiveness to provocation by methacholine at concentration equivalents in the air of about 22.4 mg/m^3 (or 12.5 ppm) similar to the concentration that produced eye irritation (25 ppm) in human volunteers as seen in the Silverman et al. (1946) study. This response is of concern and an experimental analog to asthma. This may be indicative that the same chemo-sensory response triggered both the reactiveness in the airways and eye irritation. The potentiation of methacholine-induced bronchoconstriction shows the potential of acetaldehyde at concentrations of approximately 12.5 ppm or higher to exacerbate asthma. It should be noted that the model of nebulizer used was shown to have inconsistent delivery; thus the estimate of concentration of acetaldehyde that potentiated methacholine-induced bronoconstriction is uncertain.

In summary, exposure to acetaldehyde, at concentrations as low as 25 ppm, resulted in sensory irritation in human volunteers (Silverman et al., 1946). Aerosolized acetaldehyde at concentrations equivalent to approximately 12.5 ppm potentiated bronchial hyper-responsiveness to provocation by methacholine (Myou et al., 1994). Adult asthmatics showed large inter-individual variation in PC_{20} values (59 ppm to 1200 ppm) (Prieto et al., 2000). Finally, adult asthmatics that inhaled aerosolized solutions of acetaldehyde showed increased irritation and bronchoconstriction at 293 ppm (Prieto et al., 2000). Table 5.1.1 summarizes the aerosolized acetaldehyde provocation studies in adult human volunteers.

Table 5.1.1. Summary of Aerosolized Acetaldehyde Provocation Studies in Adult Human Volunteers

Human Volunteers	Concentration in aerosol	(PC_{20})*	Reference
Japanese asthmatics	5, 10, 20, 40 mg/ml	314 ppm	Myou et al., 1993
Japanese asthmatics	5, 10, 20, or 40 mg/ml	362 ppm	Myou et al., 1994
Alcohol-sensitive Japanese asthmatics	0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, 80 mg/ml	327 ppm	Fujimura et al., 1999
Alcohol-tolerant Japanese asthmatics	0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, 80 mg/ml	500 ppm	Fujimura et al., 1999
Japanese asthmatics	0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 40, 80 mg/ml	286 ppm	Myou et al., 1995
Caucasian asthmatics	5-40 mg/ml	293 ppm	Prieto et al., 2000
Caucasian asthmatics	2.5-80 mg/ml	692 ppm	Prieto et al., 2002a
Caucasian asthmatics	2.5-80 mg/ml	631 ppm	Prieto et al., 2002b

* Values for PC_{20}, which are geometric means are converted from mg/mL of aerosolized acetaldehyde to approximate concentration in air (ppm).

5.2 Acute Toxicity to Infants and Children

No studies on the effects of acute exposure to acetaldehyde in non-adult humans were located. However, as noted above for adults, there is some evidence that following acute exposure to acetaldehyde, asthmatics are more sensitive to acetaldehyde exposure and are likely to show symptoms such as wheezing, shortness of breath, bronchoconstriction, and/or decrements in pulmonary function consistent with immediate and/or delayed bronchoconstriction. Furthermore, some asthmatics may respond with significant reductions in lung function due to the irritant effects, sensitized or not. The potential association between acetaldehyde exposure and asthma is of special concern for children because they have higher prevalence rates of asthma than adults, and their asthma episodes can be more severe due to their smaller airways. Hospitalization rates of children for asthma, especially for the first four years of life, are higher than for other age groups (Mannino et al., 1998). In addition, infants and children may have qualitatively different responses due to different target tissue sensitivities during windows of susceptibility in the developmental process.

Findings also support the view that toxic air contaminants, such as acetaldehyde, in communities in proximity to major emission sources, including both industrial and traffic sources, have adverse effects on asthma in children (Delfino et al., 2003). The average daily residential exposure to acetaldehyde in high school students living in inner-city neighborhoods of New York City and Los Angeles and living with a smoker was evaluated. The exposure concentration range measured in juveniles living with smokers was 6.3 to 14 $\mu g/m^3$ (Nazaroff, 2004). This study estimated that approximately 16 million juveniles are exposed to environmental tobacco smoke, and hence acetaldehyde by living with smokers.

5.3 Acute Toxicity to Experimental Animals

Acetaldehyde causes sensory irritation in experimental animals. Male B6C3F1 or Swiss-Webster mice were exposed to acetaldehyde in a head-only exposure chamber for 10 minutes and sensory irritation was quantified by measuring respiratory rate depression during the exposures (Steinhagen and Barrow, 1984). The respiratory rates were recorded with a plethysmograph and the average maximum decrease in respiratory rate for one minute was computed from the response of each group of animals. Five concentrations (750 to 4200 ppm) were used to construct a concentration-response curve and the RD_{50} was calculated (the concentration eliciting a 50% decrease in respiratory rate). RD_{50} values were 2932 and 2845 ppm for B6C3F1 and Swiss-Webster mice, respectively (Steinhagen and Barrow, 1984).

In a study using young adult albino male Wistar rats, acetaldehyde (nose-only) exposure resulted in an initial rapid decrease in breathing frequency during the first minutes of exposure (Cassee et al., 1996a). The minimum decrease in respiratory rate considered significant was 12%. The animals were exposed to acetaldehyde vapors for thirty minutes. The exposure concentrations were reported as 2800, 4600, and 6500 ppm for acetaldehyde. The RD_{50} for acetaldehyde in the single-compound study was calculated to be 3046 ppm (Cassee et al., 1996a).

Similarly, male F-344 rats were exposed in a head-only inhalation chamber to acetaldehyde (approximately 800 to 10,000 ppm though exact concentrations from the graph were not provided in the paper) for 10 minutes and experienced sensory irritation as measured by

reduction in respiratory rate (Babiuk et al., 1985). The RD_{50} (the level inducing a 50% reduction in respiratory breathing rate) was 2991 ppm (95% CI 2411-3825) for this study (Babiuk et al., 1985).

In addition to sensory irritation, histopathological effects have been observed after exposure to acetaldehyde. Albino, male Wistar rats, 8 weeks old, were exposed for 6 hours a day, to either one or three day exposures on consecutive days, in a nose-only inhalation chamber to acetaldehyde (750 or 1500 ppm) (Cassee et al., 1996b). Acetaldehyde exposure resulted in histopathological nasal changes with the three-day exposure group consisting of increased incidence and severity of "single-cell necrosis" in olfactory epithelium with increasing concentration. Biochemical changes consisted of concentration-dependent increases of nonprotein sulfhydryl groups in nasal respiratory epithelium with one- and three-day exposure, which was statistically significant with exposure to 1500 ppm. Activities of biotransformation enzymes (glutathione peroxidase, glutathione S-transferase, glutathione reductase, formaldehyde dehydrogenase, and nonspecific aldehyde dehydrogenase) were not affected by any of the exposures (Cassee et al., 1996b).

Acute lethality studies have also been performed with acetaldehyde. In an historical acute inhalation study in rats, groups of eight per dose were exposed to acetaldehyde vapors 7,778 to 31,667 mg/m^3 (14,000 to 57,000 ppm) for thirty minutes (Skog, 1950). The acute LD_{50} value (reported as LD_{50}) for acetaldehyde inhalation was 20,600 ppm (37,000 mg/m^3) (Skog, 1950).

Appelman et al. (1982) determined the LC_{50} for acetaldehyde using twenty male and twenty female albino Wistar rats. The animals were exposed for 4 hours in horizontally placed glass exposure cylinders with a total airflow through the cylinder of 8 l/min. Concentrations were given as the mean of 10 to 15 determinations and were as follows: 10,436, 12,673, 15,683, and 16,801 ppm. Within the first half-hour of the four-hour LC_{50} study, rats exhibited restlessness, closed eyes and labored breathing to acetaldehyde concentrations as low as 10,436 ppm. In the subacute portion of the study, rats exhibited severe dyspnoea and excitation within the first half-hour of exposure to 5000 ppm. The behavior of animals exposed to 2200 ppm or lower for six hours was unremarkable. The four-hour LC_{50} and the 95% confidence limits were calculated to be 13,300 ppm (95% CL: 11,200, 15,400) (Appelman et al., 1982).

Syrian Golden hamsters were exposed to acetaldehyde vapors for 4 hours at doses ranging from 14,450 to 17,600 ppm (26,010 to 31, 680 mg/m^3) (Kruysse et al., 1975). After one to two hours of exposure at all concentrations, the animals showed severe lacrimation, salivation, and nasal discharge. The 4-hour LC_{50} was determined to be 17,000 ppm (30,600 mg/m^3) for this study. In all exposure groups, the animals that died during exposure had convulsions. Some animals survived at all concentrations, but only after a deep narcosis and apnea (Kruysse et al., 1975).

Aldehyde dehydrogenase 2 (ALDH2) is an important enzyme that oxidizes acetaldehyde. Isse et al. (2005) compared the acute acetaldehyde toxicity between wild-type (Aldh2+/+) and Aldh2-inactive transgenic (Aldh2-/-) mice after inhalation. The null aldehyde dehydrogenase 2 (ALDH2) transgenic mice (-/-) or wild type (+/+) mice were exposed by inhalation to 5000 ppm acetaldehyde for four hours. Mice were observed at 0, 2, 20, 40, 60, 120, and 240 minutes after administration. Within the first twenty minutes, hypoactivity, crouching, bradypnea, closed eyes, and piloerection were observed in both the wild type and the knockout mice. By one hour,

the ADLH (-/-) mice were showing a staggering gait (Isse et al., 2005). This study concluded that acute acetaldehyde toxicity after inhalation is higher in aldehyde dehydrogenase 2 knockout than in wild-type mice (Isse et al., 2005).

Female CD1 mice were exposed in inhalation chambers to a target acetaldehyde exposure of 200 ppm (actual mean of 5 exposures was 180 ± 35 ppm), twice the threshold limit value, for single and multiple three-hour exposures, and then evaluated for changes in their susceptibility to experimentally induced *Streptococcus* aerosol infection and pulmonary bactericidal activity to inhaled *Klebsiella* pneumoniae after one or five days (Aranyi et al., 1986). The results showed increased pulmonary bactericidal activity in response to 200 ppm of acetaldehyde possibly by a pollutant-induced recruitment of unexposed alveolar macrophages. This study suggests that inhaled toxicants such as acetaldehyde may alter susceptibility to or severity of respiratory infection (Aranyi et al., 1986).

Table 5.3.1 summarizes the acute animal data for acetaldehyde inhalation. The data indicate that humans may be more sensitive to the acute effects of acetaldehyde than animals. For the endpoint of sensory irritation, measured as reduction in respiratory rate, the lowest RD_{50} for mice and rats were 2845 and 2991 ppm, respectively. With respect to histopathological changes, effects were observed at 1500 ppm. In the acute lethality studies, the lowest LC_{50} was 13,300 ppm in rats. In contrast, the LOAEL for human sensory irritation was reported to be 25 ppm in one historical study (Silverman et al., 1946). In addition, potentiation of methacholine-induced bronchoconstriction was shown in one study at approximately 12.5 ppm.

Table 5.3.1 Summary of Acute Studies in Experimental Animals

Endpoint	Strain/Species	Exposure	Response	Reference
Sensory irritation	B6C3F1 mice	750 to 4200 ppm for10 min	RD_{50} = 2932 ppm	(Steinhagen and Barrow 1984)
	Swiss Webster mice	750 to 4200 ppm for10 min	RD_{50} = 2845 ppm	(Steinhagen and Barrow 1984)
	F-344 rats	~800 to 10,000 ppm for 10 min	RD_{50} = 2991 ppm	Babiuk et al., 1985
	Wistar rats	2800, 4600, or 6500 ppm for 30 min	RD_{50} = 3046 ppm	Cassee et al., 1996a
Histopathological	Wistar rats	750 or 1500 ppm for 1 and 3 days	Olfactory epithelial lesions at 1500 ppm	Cassee et al., 1996b
Lethality	Rats	14,000 to 57,000 ppm for 30 min	LD_{50} = 20,600 ppm	Skog, 1950
	Wistar rats	10,436 to16,801 ppm for 4 hours	LC_{50} = 13,300 ppm	Appelman et al., 1982
	Syrian Golden hamsters	14,450 to 17,600 ppm for 4 hours	LC_{50} = 17,000 ppm	Kruysse et al., 1975
Behavioral/Other effects	Syrian Golden hamsters	14,450 for one to 2 hours	lacrimation, salivation, and nasal discharges	Kruysse et al., 1975
	Wistar rats	10,436 ppm within first 30 min	restlessness, closed eyes and labored breathing	Appelman et al., 1982
	Wistar rats	5000 ppm for 30 min	severe dyspnoea and excitation	Appelman et al., 1982
		5000 ppm for 20 minutes	crouching, brady-pnea, closed eyes, and piloerection	Isse et al., 2005a
	CD1 mice	200 ppm for 3 hours	increased pulmonary bactericidal activity	Aranyi et al., 1986

6. Chronic Toxicity of Acetaldehyde

6.1 Chronic Toxicity to Adult Humans

No studies were found for human chronic exposures. Therefore the chronic REL was based on an animal study. However, as mentioned previously, it is important to note that acetaldehyde can be produced endogenously after food intake and ethanol consumption. Therefore, certain segments of the population may be at higher risk for chronic exposure due to alcoholism or frequent drinking or smoking. Those members of the population who smoke or are consistently exposed to ETS may be at increased risk of problems related to chronic toxicity of acetaldehyde.

6.2 Chronic Toxicity to Infants and Children

No studies were found on chronic exposure of infants and children to acetaldehyde. However, we anticipate that chronic exposure to acetaldehyde may exacerbate breathing problems in infants and children with asthma.

6.3 Chronic Toxicity to Experimental Animals

Exposure to inhaled acetaldehyde produces non-carcinogenic injury including degeneration and hyperplasia in the rat respiratory tract. The nasal cavity is the primary target with nasal olfactory mucosa being more sensitive than respiratory mucosa to the effects of acetaldehyde (Morris, 1997a; b). Deposition efficiency of inhaled acetaldehyde is highly dependent on airflow rate and on the inspired concentration in rodents (Morris, 1997a; b). Pretreatment with an ALDH inhibitor reduces nasal acetaldehyde deposition rates in anesthetized rodents (Morris and Blanchard, 1992).

In a subchronic study, male and female rats were exposed to acetaldehyde (6 hr/day, 5 days/week) for four weeks to concentrations of 400, 1000, 2200, or 5000 ppm, which resulted in degeneration of olfactory nasal tissues at all concentrations. Therefore a lowest observable adverse effect level (LOAEL) for this study was 400 ppm (Table 6.3.1) (Appelman et al., 1982). Nasal respiratory tissue lesions were seen at the three highest concentrations, tracheal and laryngeal lesions were observed only at the two highest concentrations, and mild injury to the lower respiratory tract was observed only at the highest concentration. Respiratory distress (dyspnea) was noted at 5000 ppm. Subsequent 4-week exposure studies in males of the same rat species at 150 and 500 ppm, resulted in observed degeneration of olfactory nasal tissues at 500 ppm, but not in the 150 ppm exposure group (Appelman et al., 1986). Therefore, 150 ppm was designated the no observable adverse effect level (NOAEL).

Table 6.3.1: Incidence of Nasal Olfactory Tissue Effects in Rats

Degeneration of nasal olfactory epithelium	Exposure Group (ppm)						
	0	150	400	500	1000	2200	5000
Number examined	40	10	20	10	20	20	20
Number affected	2	0	16	10	20	19	20

(Appelman *et al.*, 1982)

Exposure of rats to 243 ppm (442 mg/m^3) acetaldehyde for 8 hr/day, 5 days/week for 5 weeks resulted in an "intense" nasal inflammatory reaction with olfactory epithelium hyperplasia and polymorphonuclear and mononuclear infiltration of the submucosa (Saldiva et al., 1985). Changes in pulmonary mechanics, including increased functional residual capacity, residual volume, total lung capacity, and respiratory frequency was observed, but may have been the result of mechanical damage during pulmonary function testing.

In a subchronic study, male F344 rats were exposed to acetaldehyde (6 hr/day, 5 days/week) for 13 weeks to concentrations of 0, 50, 150, 500, or 1500 ppm, which resulted in degeneration of olfactory and respiratory epithelium (Dorman et al., 2008). The lowest observable adverse effect level (LOAEL) for the endpoint of degeneration of olfactory nasal epithelium was 150 ppm for the 65-day observation (Table 6.3.2). The no observable adverse effect level (NOAEL) for degeneration of olfactory nasal epithelium was 50 ppm. For the incidence of respiratory epithelial hyperplasia (Table 6.3.3), the LOAEL was 500 ppm and the NOAEL was 150 ppm.

Table 6.3.2: Incidence of Nasal Olfactory Tissue Effects in F344 Rats at 65 Days

Degeneration of nasal olfactory epithelium	Exposure Group (ppm)				
	0	50	150	500	1500
Number examined	12	12	12	12	12
Number affected	0	0	12	12	12

From Dorman *et al.,* 2008 (supplemental data Table IV. provided by author)

Table 6.3.3. Incidence of Respiratory Epithelial Hyperplasia in F344 Rats at 65 Days

Degeneration of respiratory epithelium	Exposure Group (ppm)				
	0	50	150	500	1500
Number examined	12	12	12	12	12
Number affected	0	0	1	11	12

Dorman *et al.,* 2008 (supplemental data Table II. provided by author)

This study also examined the endpoints of incidence of respiratory epithelial inflammation and squamous metaplasia using the same dose groups and time-points (data not shown), however the degeneration of olfactory and respiratory nasal epithelia were the endpoints of interest.

In a subchronic exposure of hamsters to 0, 390, 1340, or 4560 ppm acetaldehyde 6 hr/day, 5 days/week for 90 days resulted in growth retardation, and ocular and nasal irritation in the high dose group. Histopathological changes were observed only in the respiratory tract and consisted of necrosis and inflammatory changes of the epithelium in the nasal cavity, larynx, bronchi and lungs in the high dose animals, and mild tracheal epithelial lesions in the mid-dose group. No adverse effects were observed at 390 ppm (Kruysse et al., 1975).

In a subsequent study, 36 hamsters per dose group were chronically exposed in a whole body inhalation chamber to 0, 1500, or 2500 ppm acetaldehyde for 7 hr/day, 5 days/week for 52 weeks resulting in growth retardation and hyperplasia and metaplasia of the nasal and tracheal epithelium in exposed animals (Feron et al., 1982). Rhinitis and epithelial lesions of the larynx were also noted at the highest exposure. The average concentration in the high exposure group (2500 ppm) was lowered several times during the study due to severe growth retardation to a final concentration of 1650 ppm. The authors noted that the nasal lesions were very similar to those previously seen in hamsters repeatedly exposed to 4560 ppm in the 13-week study Kruysse et al. (1975) study. Following a 26-week recovery period, the upper respiratory tract lesions were still present in high exposure animals, but were nearly or completely absent at the low exposure animals (Feron et al., 1982). However, the authors note that the acetaldehyde-induced hyperplasia and metaplasia of the nasal and laryngeal epithelium persisted and was irreversible (Feron et al., 1982).

In chronic inhalation studies, rats were exposed to 0, 750, 1500, or 3000 ppm acetaldehyde for 6 hr/day, 5 days/week for up to 28 months (Woutersen et al., 1984; Woutersen et al., 1986; Woutersen and Feron, 1987). The concentration in the high-dose group was gradually lowered over 15 months to 1000 ppm due to early mortality, respiratory distress (dyspnea) and severe growth retardation. Nasal olfactory tissue degeneration, hyperplasia, and metaplasia were seen at all exposure levels including the LOAEL of 750 ppm. A NOAEL was not determined for this study. Larynx and nasal respiratory epithelium lesions were observed at the two highest concentrations (1500 and 3000 ppm), and slight to severe rhinitis and sinusitis was observed at the highest concentration (3000 ppm). Growth retardation occurred in males of each test group and in females of the two highest concentration groups.

In a pulmonary immune response study, groups (n = 8) of non-sensitized and ovalbumin (OA)-sensitized guinea pigs were exposed to 0 or 200 ppb (360 $\mu g/m^3$) acetaldehyde or 0 or 600 ppb benzaldehyde for 6 hr/day, 5 days/week for four weeks (Lacroix et al., 2002). Two animals from each group were examined histologically and 6 animals from each group underwent bronchoalveolar lavage. Analyses of protein, PGE2 and leokotriene content, and cellularity of the BALF were reported. In sensitized animals, acetaldehyde exposure did not modify the inflammatory and allergic response to subsequent challenge with ovalbumin (OA) aerosol relative to that induced by sensitization alone. Interestingly, benzaldehyde exposure suppressed the response of sensitized guinea pigs to OA challenge. In nonsensitized guinea pigs, acetaldehyde exposure resulted in "slight irritation" (n = 2) of the lung, trachea and nasal respiratory epithelium, and induced a significant increase in the number of alveolar macrophages, but not eosinophils or neutrophils, in bronchoalveolar lavage fluid (n = 6)(Lacroix et al., 2002). There was no increase in total protein, PGE2, or leukotriene content in the BALF. Acetaldehyde exposure did not change any of these parameters in OA-sensitized animals. Limitations of the LaCroix et al. (2000) study include a lack of quantitative data for irritation and reported large variability in the concentration of acetaldehyde in the chamber atmosphere. In addition, there was no acetaldehyde-induced exacerbation of response to OA challenge in the sensitized animals. If the slight irritation seen in non-sensitized animals was exposure related, the effect would be expected to be greater in the sensitized animals than the non-sensitized animals, but there was no increase in response in acetaldehyde-exposed sensitized animals beyond sensitization alone. Finally, human data indicate exacerbation of methacholine induced bronchoconstriction after acetaldehyde exposure, yet acetaldehyde exposure did not exacerbate OA challenge in this study. Given the lack of

quantitative data on irritation, lack of exacerbation of response by acetaldehyde in OA-sensitized animals, and the inconsistency of this study with other rodent studies vis-à-vis irritation NOAELs, we decided against using this study for the 8-hour or chronic REL.

Inhaled acetaldehyde is genotoxic and is a clastogen, and induced sister chromatid exchange (Dellarco, 1988). *In vivo* and *in vitro* studies have shown that acetaldehyde can form DNA-DNA and DNA-protein crosslinks (Morris, 1997a). Acetaldehyde vapor causes chronic tissue injury and tumor formation in nasal tissues at exposure concentrations of 750 ppm or higher (Feron et al., 1982; Woutersen et al., 1986). Acetaldehyde is a Proposition 65 listed carcinogen. Carcinogenicity of acetaldehyde is discussed in the health effects assessment for identification of acetaldehyde as a Toxic Air Contaminant.

7. Developmental and Reproductive Toxicity

Both clinical and experimental studies have shown that ethyl alcohol causes developmental and reproductive toxicity. Acetaldehyde, the primary metabolite of ethyl alcohol, has been suggested as a possible etiologic agent in fetal alcohol syndrome (FAS) (Pratt, 1980; West, 1994; Eriksson, 2001). Current studies suggest that ethyl alcohol and acetaldehyde work through different mechanisms, but it is still unknown if one or both are the basis for FAS. As a small lipid soluble molecule, acetaldehyde is able to cross membranes by simple diffusion (Zorzano and Herrera, 1989a). Acetaldehyde has been shown to cross the placenta in mice and distribute to embryos (Blakley and Scott Jr., 1984b). Placental transfer occurred when acetaldehyde was administered via i.p. injection to pregnant CD-1 mice at 200 mg/kg on day 10 of gestation, and acetaldehyde was detected within the embryo within 5 minutes (Blakley and Scott Jr., 1984a; b). Maximal concentrations of acetaldehyde were also reached in the maternal blood, liver, and yolk sac in the first five minutes.

Acetaldehyde also freely crosses the placenta of Wistar rats (Zorzano and Herrera, 1989b). Following i.v. injection of acetaldehyde (10 mg/kg) to pregnant rats on gestation day 21, acetaldehyde concentrations reached peak levels within five minutes in the maternal blood, fetal blood, and amniotic fluid. Indeed, after just two minutes of maternal intravenous administration of acetaldehyde at high concentrations, it freely crosses the placenta.

Acetaldehyde has been shown to cause adverse developmental effects in some rodent species when administered in high doses via i.p. or i.v. injection. Rats were exposed 50, 75, or 100 mg/kg acetaldehyde by i.p. on gestation day 10, 11, or 12 and then sacrificed on day 21. Significant fetal resorptions and malformations were observed including: edema, microcephaly, micrognathia, micromelia, hydrocephaly, exencephaly, and hemorrhages. Somatometric measurements of fetus, crown rump length, transumbilical distance, and tail length notes severe growth retardation (Sreenathan et al., 1982). In another study in rats, after a single i.p. injection of 50, 75, or 100 mg/kg, teratogenicity, embryolethality, and growth retardation were observed (Blakley and Scott Jr., 1984a).

In vitro models have found that acetaldehyde was teratogenic to C_3H mouse embryos between 8 and 10 days of gestation after 28 hours of exposure (Thompson and Folb, 1982). Morphological parameters and DNA synthesis were measured and correlated. Eight and nine-day embryos were exposed to doses of 7.4, 19.7, or 39.4 mg/l acetaldehyde in incubation medium. The 39.4 mg/l

dose group at eight days showed a significant effect on somite count, neural tube fusion, CNS development (size and symmetry), and significant reduction in DNA synthesis. The nine-day embryos at 39.4 mg/l had increased somite count, absent heart beat, and a significant increase in limb development, while the 19.7 mg/l group had significant abnormalities in development of visceral arches, CNS development, and reduction in DNA synthesis.

Acetaldehyde significantly induced cytotoxicity *in vitro* in cultured rat embryonic midbrain cells. The levels of p53, bcl-2, and 8-OHdG were also changed by acetaldehyde treatment (Lee et al., 2005). The purpose of this study was to elucidate the molecular mechanisms involved in alcohol-induced Fetal Alcohol Syndrome (FAS) during embryo and fetal development. It is not clear whether the observed toxicity associated with FAS is due to direct exposure to ethanol, to its metabolite(s) (e.g. acetaldehyde) or to both.

Both acetaldehyde and ethanol significantly inhibited the gonadotropin-stimulated biosynthesis of testosterone, and acetaldehyde and was 4,000 times more potent than ethanol *in vitro* in enzymatically dispersed cells. Testicular steroidogenesis was blocked by acetaldehyde selectively, specifically inhibiting the conversion of androstenedione to testosterone (Cicero and Bell, 1980; Cicero et al., 1980a; Cicero et al., 1980b). As little as 50 μM acetaldehyde was effective in suppressing testicular steroidogenesis; however, cell viability was unaffected.

8. Derivation of Reference Exposure Levels

8.1 Acetaldehyde Acute Reference Exposure Level

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 in the Technical Support Document).

Numerous studies on adult humans with and without asthma, investigated provocation with acetaldehyde solutions in saline (Table 5.1.1), which resulted in significant pulmonary decrements and more so in asthmatics. The study by Prieto et al. (2000) was selected for development of the acute REL as it investigated short-term exposure of human volunteers to aerosolized acetaldehyde solutions.

Sixty-one asthmatic subjects were used to determine the concentration of acetaldehyde producing a 20% fall (PC_{20}) in Forced Expiratory Volume in one second (FEV_1) using ascending doses (5 to 40 mg/ml) of aerosolized acetaldehyde solutions. The geometric mean concentration of PC_{20} for the sixty-one subjects was 17.55 mg/ml of acetaldehyde and the values ranged from 1.96 to 40 mg/mL (Prieto et al., 2000). The 95% confidence interval of the geometric mean was (4.72-38.3 mg/ml) (Prieto, 2008). Therefore, the lower bound of the geometric 95% CI was 4.72 mg/ml, which was converted to ppm, and was the value used as the point of departure for the acute REL derivation (Prieto, 2008). The Hudson 1720 nebulizer was operated at 6 liters air/minute for 2 to 4 minutes with an acetaldehyde solution output of 0.18 ml/minute. Therefore, the lower 95% confidence interval of 4.72 mg/ml corresponds to approximately 142 mg/m^3 (about 79 ppm).which was used as the LOAEL for the acute REL determination.

Study	Prieto et al., 2000
Study population	61 adult asthmatic human volunteers
Exposure method	Inhalation by nebulizer
Exposure continuity	
Exposure duration	2-4 minutes
Critical effects	Bronchoconstriction, PC_{20} >20% drop in FEV_1
LOAEL	142 mg/m^3 (79 ppm)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	10 (severe effect, no NOAEL)
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (inter-individual variation)
Toxicodynamic (UF_{H-d})	30 (asthma exacerbation in children, hyper-responsiveness to methacholine)
Cumulative uncertainty factor	300
Reference Exposure Level	**470 μg/m^3 (260 ppb)**

An uncertainty factor of ten is associated with the use of a LOAEL for severe effects in the absence of a NOAEL (see Section 4.4.5 of the TSD). The key study used to determine the acute REL was a human study, therefore the interspecies uncertainty factor, toxicokinetic (UF_{A-k}) and toxicodynamic (UF_{A-d}) components were each assigned the default value of one.

For the toxicokinetic component of the intraspecies uncertainty factor (UF_{H-k}) a value of one was used since sensory irritation is not expected to involve large toxicokinetic differences among individuals, and the effects are largely confined to the site of contact, in this case, the eyes, nose, and upper respiratory tract, with negligible or no systemic effects. The deposition kinetics of reactive gases is generally thought not to be greatly different between adults and children. Because of this, a value of one is used for the kinetic component of the intraspecies uncertainty factor (UF_{H-k}), rather than a more extended values of √10 or ten used where metabolic processes also contribute to inter-individual variability.

The toxicodynamic component of the intraspecies uncertainty factor UF_{H-d} was assigned an increased value of 30 for the acute REL determination due to multiple lines of evidence. A portion of the UF_{H-d} is applied to account for the potential greater susceptibility of children. The respiratory irritant effect of acetaldehyde, with documented potential to exacerbate asthma, is an effect with the potential to differentially impact infants and children. Myou et al., 1994 demonstrated hyper-responsiveness to methacholine after provocation with a sub-threshold dose of aerosolized acetaldehyde at concentrations equivalent to approximately 12.5 ppm. Additional studies have also shown that adult asthmatics are more sensitive to the irritative properties of

inhaled aerosolized acetaldehyde solutions, which significantly decreased their forced expiratory volume in one second (FEV_1) by more than 20% (Myou et al., 1993; Myou et al., 1994, Fujimura et al., 1999, Prieto et al., 2002a; Prieto et al., 2002b). Finally, alcohol sensitive asthmatics had a selective hyper-responsiveness to acetaldehyde (Myou et al., 1993; Myou et al., 1994; Fujimura et al., 1999).

Myou et al. (1994) also observed that aerosolized acetaldehyde potentiated bronchial hyper-responsiveness to provocation by methacholine at concentration equivalents in the air (22.4 mg/m^3 or 12.5 ppm) similar to the concentration that produced eye irritation (25 ppm) in human volunteers as seen in the Silverman et al. (1946) study. This response is of concern and an experimental analog to asthma. This may be indicative that the same chemo-sensory response triggered both the reactivity in the airways and eye irritation. The potentiation of methacholine-induced bronchoconstriction shows the potential of acetaldehyde at concentrations of approximately 12.5 ppm or higher to exacerbate asthma. Of note however, some uncertainty is associated with the use of a DeVillbis nebulizer, which has been shown to have considerable variability in aerosol output and delivered dose (Hollie et al., 1991).

In conclusion, using the LOAEL of 142 mg/m^3 (79 ppm) for bronchoconstriction from Prieto et al. (2000), divided by the cumulative uncertainty factor of 300, an acute reference exposure level (REL) for acetaldehyde was determined to be 470 $\mu g/m^3$ (260 ppb). This level is considered safe for infants and children during an acute exposure period.

Strengths of the Prieto et al. (2000) study include: the study was performed in human subjects, had good experimental design, and a large sample size (n = 61 adult asthmatics) compared to the other aerosolized acetaldehyde provocation studies, and had an endpoint (bronchoconstriction) of interest and concern. Limitations of the Prieto et al. (2000) study include very short exposure periods of 2-4 minutes and the use of aerosolized acetaldehyde solutions in saline. In view of possible but unquantified differences between deposition from an aqueous aerosol and from the gas phase, and resulting differences in dose received by bronchial tissues, there is uncertainty involved in converting the concentration values from mg acetaldehyde/ml solution to an equivalent concentration in air. In provocation studies by other groups, a DeVilbiss nebulizer was used, which has been shown to have considerable variability in aerosol output (Hollie et al., 1991). However, the Prieto study used a Hudson 1720 nebulizer, which is considered to be more consistent.

In a supporting study, Silverman et al. (1946) investigated eye irritation in non-asthmatic adults after acetaldehyde whole body exposure. Upper respiratory tract, nose, throat, and bronchial irritation typically followed that effect closely. Exposure to 50 ppm for 15 minutes caused moderate eye irritation in all subjects, whereas 25 ppm caused complaints of slight eye irritation in an unspecified number of volunteers. Nose and throat irritation and transient conjunctivitis were seen at concentrations of 200 ppm or greater. The Silverman et al. (1946) study had a LOAEL of 25 ppm for slight eye irritation, but a NOAEL was not determined.

Study	Silverman et al., 1946
Study population	24 adult human volunteers
Exposure method	Whole body
Exposure continuity	
Exposure duration	15 minutes
Critical effects	Eye and upper respiratory tract irritation
LOAEL	45 mg/m^3 (25 ppm)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	not applied (sensory irritation, no Haber's Law adjustment)
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	6 (default: mild effect, no NOAEL)
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	60
Reference Exposure Level	**750 μg/m^3 (420 ppb)**

In this supporting study, the output (acetaldehyde vapor) is sent generally into an environmental chamber in an effort to mimic real-life exposures and the subject's nose, respiratory tract, eyes, and uncovered skin are concomitantly exposed to the chemical stimulus (Silverman et al., 1946). Generally speaking, the lowest concentration of an irritant that can be discerned by sniffing or by ocular exposure is considered to be the threshold for irritation (Doty et al., 2004). As a general rule, most volatile chemicals that are capable of eliciting irritative sensations (e.g., via the trigeminal nerve) can also elicit an odor (via CN I); furthermore, the odor is often evoked at concentrations one or more orders of magnitude below those that evoke irritation. For most volatile chemicals, ocular irritation is equivalent in sensitivity to nasal irritation in humans with thresholds of equivalent magnitude (Cometto-Muniz and Cain, 1995; 1998; Cometto-Muniz et al., 1998; Cometto-Muniz et al., 1999; 2001; 2002; Doty et al., 2004).

The trigeminal nerve, which gathers sensory signals from the nasal mucosa amongst several other places, appears to be the only sensory nerve pathway directly involved with the respiratory response to inhaled irritants. In rodents, a reflex decrease in respiratory rate is observed after the initial sensory irritation (Bos et al., 2002); the human response is more complex in its expression although similar in neurological mechanism.

A default uncertainty factor of six is associated with the use of a LOAEL for mild effects in the absence of a NOAEL (see Section 4.4.5 of the TSD). The study was performed in humans, therefore the interspecies uncertainty factor, toxicokinetic (UF_{A-k}) and toxicodynamic (UF_{A-d}) components were each assigned the default value of one. Eye irritancy appears to be more a

function of concentration rather than duration of exposure (Yang et al., 2001), so no time correction factor was applied.

For the toxicokinetic component of the intraspecies uncertainty factor (UF_{H-k}) a value of one was used since sensory irritation is not expected to involve large toxicokinetic differences among individuals, and the effects are largely confined to the site of contact, in this case, the eyes, nose, and upper respiratory tract, with negligible or no systemic effects. The deposition kinetics of reactive gases is generally thought not to be greatly different between adults and children. Because of this, a value of one is used for the kinetic component of the intraspecies uncertainty factor (UF_{H-k}), rather than a more extended values of $\sqrt{10}$ or ten used where metabolic processes also contribute to inter-individual variability.

A toxicodynamic uncertainty factor (UF_{H-d}) of ten was used to account for the potential greater susceptibility of children. While ocular irritation is not expected to be substantially different between children and adults, the respiratory irritant effect, with documented potential to exacerbate asthma, is clearly an effect with the potential to differentially impact infants and children. The toxicodynamic component of the intraspecies uncertainty factor UF_{H-d} is therefore assigned an increased value of ten to account for potential asthma exacerbation. As mentioned earlier, asthmatics are more sensitive to the irritative properties of inhaled aerosolized acetaldehyde solutions, which significantly decreased their forced expiratory volume in one second (FEV_1) by more than 20% (Prieto et al., 2000; Prieto et al., 2002b). And, alcohol sensitive asthmatics had a selective hyper-responsiveness to acetaldehyde (Myou et al., 1993; Myou et al., 1994; Fujimura et al., 1999). These considerations are applied equally to the acute, 8-hour and chronic REL.

Limitations with the Silverman et al. (1946) study include: small sample size, subjective and non-quantitative measure of irritation, absence of a clear description of exposure method and experimental procedure, which was further unsubstantiated by lack of a clear experimental procedure.

In conclusion, using the LOAEL of 45 mg/m^3 (25 ppm) for eye irritation from Silverman et al. (1946), divided by the cumulative uncertainty factor of 60, an acute reference exposure level (REL) for acetaldehyde was determined to be 750 $\mu g/m^3$ or 420 ppb for the endpoint of eye irritation. Therefore, the acute REL calculated using the key study of Preito et al. (2000) of 470 $\mu g/m^3$ or 260 ppb would also be protective for eye irritation.

8.2 Acetaldehyde 8-Hour Reference Exposure Level

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the Technical Support Document).

Bronchoconstriction, eye irritation and nasal mucosal histopathology are all legitimate concerns for the 8-hour REL and occur in a broadly similar concentration range over the relevant time scale. The repeated nature of an 8-hour REL makes use of the acute studies inappropriate. Therefore, the 8-hour REL was derived using the subchronic animal study (Appelman et al., 1982; 1986) in rats exposed to acetaldehyde six hours per day, five days per week for four

weeks. Incidence of degeneration of nasal olfactory epithelium was the most sensitive end-point. These data are supported by Dorman et al. (2008) who reported endpoints of degeneration of the nasal olfactory and respiratory epithelia.

Study	Appelman et al., 1982; 1986
Study population	Wistar rats (10-40 animals/group)
Exposure method	Inhalation exposure to 0, 273, 728, 910, 1820, 4004, 9100 mg/m^3 (0, 150, 400, 500, 1000, 2200, or 5000 ppm)
Exposure continuity	6 hours per day, 5 days/week
Exposure duration	4 weeks
Critical effects	Degeneration of olfactory epithelium
LOAEL	720 mg/m^3 (400 ppm)
NOAEL	270 mg/m^3 (150 ppm)
Benchmark Concentration (BMC$_{05}$) (using continuous model)	178 mg/m^3 (99 ppm)
Human equivalent concentration	242.1 mg/ m^3 (134.6 ppm)(99 ppm* 1.36 (DAF) Teeguarden et al. (2008)
Time-adjusted exposure	86.5 mg/m^3 (48.1 ppm) = (134.6*6/24*20/10*5/7)
LOAEL uncertainty factor (UF$_L$)	1
Subchronic uncertainty factor (UFs)	√10 (exposure 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF$_{A-k}$)	1 (interspecies PBPK model for acetaldehyde)
Toxicodynamic (UF$_{A-d}$)	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF$_{H-k}$)	√10 (inter-individual variation)
Toxicodynamic (UF$_{H-d}$)	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	300
Reference Exposure Level	**300 µg/m^3 (160 ppb)**

The animal studies by Appelman et al. (1982; 1986) used subchronic exposure of Wistar rats to acetaldehyde for six hours per day, 5 days per week, for four weeks. Incidence of degeneration of nasal olfactory epithelium was the most sensitive end-point. The animal study has a histopathological endpoint for which there is a presumption of Haber's law (C x t) cumulation, at least over moderate timeframes. The time adjustment for an 8-hour REL used is 6 h/24 h x 20 m3/10 m3, rather than 6 h/8 h, because we assume that the 8 hours includes the active waking period when an adult inhales 10 m3 of air, i.e. half the daily total intake of 20 m3.

The 8-hour REL was determined using the Benchmark Dose (BMDS) program developed by the U.S. EPA (2003). The BMC$_{05}$ is defined as the 95% lower confidence limit of the concentration expected to produce a response rate of 5%. The animal data from the Appelman et al. (1982; 1986) studies were used to develop a BMC$_{05}$ for acetaldehyde.

The male and female data were analyzed both together and separately (Table 8.2.1). The study with exposure concentrations of 150 and 500 ppm used only males. Data on incidence of degeneration of olfactory epithelium were converted to a continuous data set ranked by severity

of effect (Table 8.2.1). The means and standard deviations at each dose-group are shown, which were calculated from the severity grading of individual animals in each dose group. Each severity category had a name and a corresponding value assigned: no effect = zero, minimal = one, slight = two, moderate = three, marked = 4, moderate with hyperplasia = 5, severe with hyperplasia = 6, and very severe with hyperplasia =7. The means and standard deviations for each dose group were entered into the BMDS program using continuous modeling. The Hill and Polynomial models in the BMDS program gave the best fit to the data (Table 8.2.2). The mean of the three models that best fit the data was calculated to be 99 ± 1.20 ppm and used as the BMC_{05}.

Table 8.2.1. Incidence of Degeneration of Olfactory Epithelium using Weighted Means by Severity[1] .

	Males			**Females**[2]		
Dose (ppm)	Number	Mean	Stdev	Number	Mean	Stdev
0	30	0.07	0.25	10	0	0
150	10	0	0			
400	10	2.6	1.17	10	0.9	0.74
500	10	2.5	0.97			
1000	10	2.8	0.63	10	3.6	0.70
2200	10	5.3	2.21	10	5.1	1.91
5000	10	6.7	0.67	10	6.9	0.32

[1]Severity categories: no effect=0; minimal=1; slight=2; moderate=3; marked=4; moderate w/ hyperplasia=5; severe w/ hyperplasia=6; and very severe w/ hyperplasia=7.
[2] In the 150 and 500 ppm dose groups, only male animals were used.

Table 8.2.2. BMDS Results Modeling Incidence of Degeneration of Nasal Olfactory Epithelium Using Weighted Means by Severity in Rats Using a Continuous Model.

Method	BMC_{05}*	BMC*	P-value	AIC
Hill Model	100	205	0.07	55.96
Polynomial (2°)	101	126	0.02	56.18
Polynomial (3°)	97	165	0.03	55.95

* BMC_{05} and BMC are in units of ppm. Source data from Appelman et al. (1982; 1986)

The standard Human Equivalent Concentration (HEC) adjustment using an RGDR was not used for the dosimetric interspecies extrapolation. Instead, species information based on pharmacokinetic modeling for toxicants that result in specific nasal olfactory tissue damage was applied for interspecies extrapolation of acetaldehyde toxicity (Teeguarden et al., 2008). Dosimetry data for the nasal olfactory epithelium shows that the rat is more efficient in scrubbing organic vapors in this region of the nasal cavity than humans (Frederick et al., 1998; Frederick et al., 2001). Consequently, rats receive a similar, or greater, tissue dose of inhaled organic vapors than humans in the olfactory epithelium. Sensitivity to acetaldehyde of the rat olfactory epithelium is a major factor for olfactory tissue damage, even though the specific activity of aldehyde dehydrogenase is greater in the respiratory epithelium (Bogdanffy et al., 1998; Stanek and Morris, 1999). The interspecies adjustment also takes into account differences in the deposition of inhaled vapors and breathing rates. While rodents are obligate nose

breathers, humans are not, which has implications for exposure of nasal tissues. Other factors when extrapolating toxicity findings from rodents to humans include dosimetry, nasal anatomy and airflow dynamics, target tissue metabolism, species differences in gross anatomy, distribution of nasal airway epithelia, and distribution and composition of mucous secretory products (Feron et al., 2001).

The dosimetric adjustment factor (DAF) was derived based on a physiologically based pharmacokinetic (PBPK) model of rat and human nasal tissues constructed for acetaldehyde (see Section 4.4.7.2.2 of the TSD). The rodent model was developed using published metabolic constants and calibrated using upper-respiratory-tract acetaldehyde extraction data (Teeguarden et al., 2008). The human nasal model incorporated previously published tissue volumes, blood flows, and acetaldehyde metabolic constants. The acetaldehyde upper airway PBPK model is structurally the same as the inhalation vinyl acetate model consisting of the nasal cavity, nasopharynx, and larynx (Plowchalk et al., 1997; Bogdanffy et al., 1999; Bogdanffy et al., 2001). The computational fluid dynamic model compartmentalizes the nasal cavity by specific tissue type and location. The rat nasal cavity model has five major compartments, and the human model structure has four. Equations for acetaldehyde concentration, flux, and pH in rats and humans were provided with the model (Teeguarden et al., 2008) . In addition a sensitivity analysis was performed to incorporate humans with ALDH2 polymorphisms into the model. The respiratory and olfactory epithelial tissue acetaldehyde concentrations were determined to be largely linear functions in both species. The impact of the ALDH2 polymorphisms was deemed negligible and not a significant contributor to acetaldehyde metabolism in the nasal tissues (Teeguarden et al., 2008).

OEHHA determined the DAF using the acetaldehyde concentration metric by calculating the ratio of acetaldehyde concentration values reported for the rat (8.41) and human (6.20), which equaled 1.36. This ratio was then multiplied by the NOAEL to obtain a human equivalent concentration (HEC) (see REL summary table for calculation) (Teeguarden et al., 2008).

Since a PBPK model specifically for acetaldehyde was used, the toxicokinetic component of the interspecies uncertainty factor $UF_{A\text{-}k}$ was assigned a value of one. In addition, since acetaldehyde exerts mainly a localized effect on nasal olfactory epithelium, toxicokinetics including distribution and metabolism play less of a key role, the extent of likely interspecies variation is likely less than the default of $\sqrt{10}$.

The LOAEL uncertainty factor (UF_L) of one was chosen, since both a LOAEL and NOAEL were determined in the key studies (Appelman et al., 1982; Appelman et al., 1986), and the benchmark approach was used to determine the 8-hour REL. In addition, the subchronic uncertainty factor (UFs) was assigned a value of $\sqrt{10}$ since the 8-hour REL is based on anticipated repeated exposures over a longer period of time than the study duration of four weeks.

The toxicodynamic portion of the interspecies uncertainty factor ($UF_{A\text{-}d}$) is $\sqrt{10}$ because the key studies are in non-primates and data on toxicodynamic interspecies differences are not available.

An uncertainty factor ($UF_{H\text{-}k}$) of $\sqrt{10}$ was used to account for intra-individual toxicokinetic variation. The intraspecies uncertainy factor was selected because acetaldehyde is a reactive substance that produces lesions at the point of contact with the tissue, therefore there would be

less variability to take into account for children versus adults. However, data are not available for the impact of ALDH2 deficiency on olfactory tissue lesions. One study does indicate that in Japanese alcohol-sensitive asthmatics versus alcohol-insensitive asthmatics, PC_{20} geometric mean values were 330 ppm versus 500 ppm, respectively, but their ALDH2 status was unknown (Fujimura et al., 1999).

The toxicodynamic uncertainty factor (UF_{H-d}) of 10 was used to account for the potentially greater susceptibility of children and asthmatics. The resulting cumulative uncertainty factor was calculated as 300 and used to determine the 8-hour REL of the experimental animal study. The 8-hour REL with the endpoint of degeneration of olfactory epithelium in rats was calculated to be 300 $\mu g/m^3$ (160 ppb).

Dorman et al. (2008) conducted a 13-week study in male F344 rats (n=12 per group) with acetaldehyde exposures of 0, 50, 150, 500, or 1500 ppm. They reported degeneration of olfactory and respiratory epithelium (Dorman et al., 2008). The LOAEL and NOAEL for the endpoint of degeneration of olfactory nasal epithelium were 150 and 50 ppm, respectively for the observations at 65 days (Table 6.3.2). Benchmark concentration analysis was performed on the data and several models provided a BMC_{05} in close agreement with the NOAEL (quantal linear BMC_{05} = 45.3 ppm and probit BMC_{05} = 48.3 ppm), but statistically were not as reliable due to the small sample size and dose spacing. Adjusting the NOAEL using the dosimetric adjustment factor (DAF) of 1.36, as described previously, based on the PBPK model for acetaldehyde (Teeguarden et al., 2008), yielded a NOAEL of 68 ppm. Thus the BMC_{05} value from the Dorman study and also the LOAEL and NOAEL values from the same study are supportive of the 8-hour REL determined from the data of Appelman et al. (1982; 1986).

For the incidence of another endpoint reported by Dorman et al. (2008), respiratory epithelial hyperplasia, benchmark concentration modeling was performed on the 65-day exposure data (Table 6.3.3). The Probit model yielded the best result with a BMC_{05} of 100 ppm, which is in good agreement with the BMC_{05} of 99 ppm from the Appelman study and is therefore also supportive of the derived 8-hour REL.

The Dorman et al (2008) study was not used for determination of the 8-hour REL due to its small sample size and the response rate rising from 0% to 100% in the olfactory epithelium data. This creates uncertainty in determination of a "true NOAEL" and an inability to use benchmark dose modeling in determination of the REL due to lack of an adequate fit of the model to the data. Another limitation of the Dorman study was the length of the study was 12.5% of the test animal's lifetime, which borders the criteria for subchronic and chronic (12% of lifetime). With the Appelman studies, not only could the benchmark dose be determined for incidence, but also the provision of severity grading data for each individual animal allowed for continuous BMDS analysis, which provided a better dose-response and low-end extrapolation of the data.

Eye irritation and nasal mucosal histopathology are both legitimate concerns for the 8-hour REL for acetaldehyde and occur in a broadly similar concentration range over the relevant time scale. However, repeated 8-hour exposures could result in tissue damage. Therefore, the REL (300 $\mu g/m^3$ (160 ppb)) using the animal study with a histopathological endpoint was used. The experimental animal study used as the basis for the 8-hour REL, with an endpoint of degeneration of nasal olfactory epithelium, would also be protective of the human sensory

response since the acute REL derived from the Silverman et al. (1946) human study is higher. The animal study was chosen because it was a well-conducted study with adequate dose groups and a time-period relevant for the 8-hour REL. In addition, using benchmark dose and PBPK modeling decreased the uncertainty associated with the REL derivation compared with using the traditional NOAEL/LOAEL and HEC (with an RGDR) procedures.

8.3 Acetaldehyde Chronic Reference Exposure Level

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

Study	Appelman et al., 1982; 1986
Study population	Wistar rats (10-40 animals/group)
Exposure method	Inhalation exposure to 0, 273, 728, 910, 1820, 4004, 9100 mg/m3 (0, 150, 400, 500, 1000, 2200, or 5000 ppm)
Exposure continuity	6 hours per day, 5 days/week
Exposure duration	*4 weeks*
Critical effects	Degeneration of olfactory epithelium
LOAEL	720 mg/m^3 (400 ppm)
NOAEL	270 mg/m^3 (150 ppm)
Benchmark Concentration (BMC_{05}) (using continuous model)	178 mg/m^3 (99 ppm)
Human equivalent concentration	242.1 mg/ m^3 (134.6 ppm)(= 99 * 1.36 (DAF) Teeguarden et al. (2008)
Time-adjusted exposure	43.2 mg/m^3 (24 ppm) = (134.6*6/24*5/7)
LOAEL uncertainty factor (UF_L)	1
Subchronic uncertainty factor (UFs)	√10 (exposure 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (intraspecies PBPK model for acetaldehyde)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (inter-individual variation)
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	300
Reference Exposure Level	**140 μg/m^3 (80 ppb)**

The chronic REL was based on four-week exposure data in rats from Appelman et al., (1982, 1986), and supported by Saldiva et al., (1985); Woutersen et al., (1986, 1984); and (Woutersen and Feron, 1987), which included a 28-month chronic study in rats. Incidence of degeneration of nasal olfactory epithelium was the most sensitive end-point. The proposed chronic REL was estimated by a benchmark concentration modeling approach using the continuous polynomial and Hill models of analysis (U.S. EPA, 2003) as previously described in detail in Section 8.2. The average experimental exposure data were adjusted to reflect chronic exposure. Table 8.2.1 shows the data expressed as the mean and standard deviation of the degeneration of nasal

olfactory epithelium by severity for each dose group, which were the data used for the BMDS model. As shown in Table 8.2.2, three models were selected that best fit the data and their mean and standard deviation was 99 ± 1.20 ppm and therefore used as the BMC_{05}.

As described in detail in Section 8.2, OEHHA used a dosimetric adjustment factor (DAF) for acetaldehyde of 1.36 based on the PBPK model for acetaldehyde developed by Teeguarden et al. (2008). The limited uncertainty associated with this assumption is reflected in the use of the toxicokinetic component of the interspecies uncertainty factor $UF_{A\text{-}k}$ equaling one since the model was specific for acetaldehyde.

The animal studies by Appelman et al. (1982; 1986) used subchronic exposure of Wistar rats to acetaldehyde for six hours per day, 5 days per week, for four weeks. Incidence of degeneration of nasal olfactory epithelium was the most sensitive endpoint.

The LOAEL uncertainty factor (UF_L) of one was chosen, since both a LOAEL and NOAEL were determined in the key studies (Appelman et al., 1982; Appelman et al., 1986), and the benchmark approach was used to determine the chronic REL.

The subchronic uncertainty factor (UFs) was assigned a value of √10 since the chronic REL is representative of exposures over a lifetime, and because the supporting chronic study (Woutersen et al., 1986) didn't give a dramatic increase in injury compared to the four-week studies by Appelman et al., (1982; 1986). In addition, Saldiva et al., (Saldiva et al., 1985) observed "intense" nasal lesions in rats exposed to 442 mg/m^3 (243 ppm) for slightly longer exposure durations than that used by Appelman et al., (1982; 1986).

The value of one was chosen for the toxicokinetic component of the interspecies uncertainty factor ($UF_{A\text{-}k}$) since a DAF from a PBPK model for acetaldehyde was used, which adequately incorporates the differences between humans and rodents (Teeguarden et al., 2008). The toxicodynamic portion of the interspecies uncertainty factor ($UF_{A\text{-}d}$) is √10 because the key studies are in non-primates and data on toxicodynamic interspecies differences are not available.

Intraspecies variability can be as much as a factor of 1,000-fold for VOCs measured in human subjects (Fenske and Paulson, 1999). An uncertainty factor ($UF_{H\text{-}k}$) of √10 was used to account for intra-individual toxicokinetic variation. The intraspecies uncertainty factor was selected because acetaldehyde is a reactive substance that produces lesions at the point of contact with the tissue, therefore there would be less kinetic variability to take into account for children versus adults. The toxicodynamic uncertainty factor (UF_{Hd}) of 10 was used to account for the potentially greater susceptibility of children and asthmatics.

The resulting cumulative uncertainty factor was calculated to be 300 and used to determine the chronic REL of the experimental animal study. The chronic REL with the endpoint of degeneration of olfactory epithelium in rats was calculated to be 140 $\mu g/m^3$ (80 ppb).

The current chronic RfC for acetaldehyde determined by the U.S. EPA and based on Appelman et al. (1982; 1986) is 9 $\mu g/m^3$ (5 ppb) and is within the range of normal human breath acetaldehyde concentrations of 0.7 to 11.0 $\mu g/m^3$ (0.4 to 6.1 ppb). OEHHA's proposed chronic REL of 140 $\mu g/m^3$ (76 ppb) is above the range of human breath concentrations of acetaldehyde,

but is mostly exceeded when humans consume significant amounts of alcohol, resulting in human breath concentrations ranging from 200 to 2200 μg/m^3. Thus, frequent alcohol use and abuse by humans is a major source of acetaldehyde exposure to the airway tissue that can exceed the chronic REL.

The LOAEL of 750 ppm from the chronic exposure data by Woutersen et al., (1984, 1986) and Woutersen and Feron (1987) produced similar injuries and was confined to the nasal olfactory epithelium as the LOAEL of 400 ppm from the 4-week Appelman studies. Thus, the subchronic UF was kept at √10, to account for similar findings from the chronic studies.

Analyses were also performed on the incidence of respiratory epithelial changes using the LOAEL from the chronic rat studies, although it was a less sensitive end-point (Woutersen et al., 1984, 1986; Woutersen and Feron 1987). The 100% response rate at the LOAEL combined with the lack of a NOAEL prevented the chronic studies from becoming the basis of the REL.

Significant strengths for the chronic REL include: (1) the use of a well conducted repeated exposure study with histopathological analysis and (2) independent studies demonstrating comparable key effects (nasal lesions) in experimental animals. However, major areas of uncertainty are the lack of adequate human chronic inhalation dose-response data in adults and children, and inadequate long-term inhalation animal data, therefore a subchronic animal study was used.

8.4 Acetaldehyde as a Toxic Air Contaminant

Acetaldehyde was identified by the ARB as a toxic air contaminant (TAC) in accordance with section 39657(b) of the California Health and Safety Code (Title 17, California Code of Regulations, section 93001) (CCR, 2007). In view of the potential of acetaldehyde to exacerbate asthma (Section 5.1, 5.2), and the differential impacts of asthma on children including higher prevalence rates, coupled with widespread exposure (e.g., indoors from exposure to environmental tobacco smoke, and outdoors due to numerous emissions sources), OEHHA recommends that acetaldehyde be identified as a toxic air contaminant (TAC) that may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

9. References

Appelman LM, Woutersen RA and Feron VJ (1982). Inhalation toxicity of acetaldehyde in rats. I. Acute and subacute studies. Toxicology 23(4): 293-307.

Appelman LM, Woutersen RA, Feron VJ, Hooftman RN and Notten WR (1986). Effect of variable versus fixed exposure levels on the toxicity of acetaldehyde in rats. J Appl Toxicol 6(5): 331-336.

Aranyi C, O'Shea WJ, Graham JA and Miller FJ (1986). The effects of inhalation of organic chemical air contaminants on murine lung host defenses. Fundam Appl Toxicol 6(4): 713-720.

Babiuk C, Steinhagen WH and Barrow CS (1985). Sensory irritation response to inhaled aldehydes after formaldehyde pretreatment. Toxicol Appl Pharm 79(1): 143-149.

Baselt RC and Cravey RH (1989). Disposition of Toxic Drugs and Chemicals in Man. 3rd. Edition. Chicago (IL): Year Book Medical Publishers Inc.

Beall JR and Ulsamer AG (1981). Toxicity of volatile organic compounds present indoors. Bull N Y Acad Med 57(10): 978-996.

Blakley PM and Scott Jr. WJ (1984a). Determination of the proximate teratogen of the mouse fetal alcohol syndrome. 1. Teratogenicity of ethanol and acetaldehyde. Toxicol Appl Pharm 72(2): 355-363.

Blakley PM and Scott Jr. WJ (1984b). Determination of the proximate teratogen of the mouse fetal alcohol syndrome. 2. Pharmacokinetics of the placental transfer of ethanol and acetaldehyde. Toxicol Appl Pharm 72(2): 364-371.

Bogdanffy MS, Plowchalk DR, Sarangapani R, Starr TB and Andersen ME (2001). Mode-of-action-based dosimeters for interspecies extrapolation of vinyl acetate inhalation risk. Inhal Toxicol 13(5): 377-96.

Bogdanffy MS, Sarangapani R, Kimbell JS, Frame SR and Plowchalk DR (1998). Analysis of vinyl acetate metabolism in rat and human nasal tissues by an in vitro gas uptake technique. Toxicol Sci 46(2): 235-246.

Bogdanffy MS, Sarangapani R, Plowchalk DR, Jarabek A and Andersen ME (1999). A biologically based risk assessment for vinyl acetate-induced cancer and noncancer inhalation toxicity. Toxicol Sci 51(1): 19-35.

Bos PMJ, Busschers M and Arts JHE (2002). Evaluation of the sensory irritation test (Alarie test) for the assessment of respiratory tract irritation. J Occup Environ Med 44(10): 968-976.

Brown VM, Crump DR, Gardiner D and Gavin M (1994). Assessment of a passive sampler for the determination of aldehydes and ketones in indoor air. Environ Technol 15: 679-685.

Burt J, Nelson NA and Kaufman JD (1996). Effects of ventilation flushout on indoor air quality in a newly constructed office building. Appl Occup Environ Hyg 11(6): 546-552.

CARB. (1993). *Acetaldehyde as a Toxic Air Contaminant. Part B, Health Assessment*. Sacramento (CA): California Air Resources Board, California Environmental Protection Agency.

Casanova-Schmitz M, David RM and Heck HD (1984). Oxidation of formaldehyde and acetaldehyde by NAD^+-dependent dehydrogenases in rat nasal mucosal homogenates. Biochem Pharmacol 33(7): 1137-1142.

Cassee FR, Arts JH, Groten JP and Feron VJ (1996a). Sensory irritation to mixtures of formaldehyde, acrolein, and acetaldehyde in rats. Arch Toxicol 70(6): 329-337.

Cassee FR, Groten JP and Feron VJ (1996b). Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde, and acrolein. Fundam Appl Toxicol 29(2): 208-218.

CCR (2007). California Code of Regulations Section 93001 Hazardous Air Pollutants Identified as Toxic Air Contaminants. Sacramento, CA: California Office of Administrative Law. 8-20-07. http://ccr.oal.ca.gov/linkedslice/default.asp?SP=CCR-1000&Action=Welcome.

Cicero TJ and Bell RD (1980). Effects of ethanol and acetaldehyde on the biosynthesis of testosterone in the rodent testes. Biochem Biophys Res Commun 94(3): 814-819.

Cicero TJ, Bell RD and Badger TM (1980a). Acetaldehyde directly inhibits the conversion of androstenedione to testosterone in the testes. Adv Exp Med Biol 132: 211-217.

Cicero TJ, Bell RD, Meyer ER and Badger TM (1980b). Ethanol and acetaldehyde directly inhibit testicular steroidogenesis. J Pharmacol Exp Ther 213(2): 228-233.

Cometto-Muniz JE and Cain WS (1995). Relative sensitivity of the ocular trigeminal, nasal trigeminal and olfactory systems to airborne chemicals. Chem Senses 20(2): 191-198.

Cometto-Muniz JE and Cain WS (1998). Trigeminal and olfactory sensitivity: Comparison of modalities and methods of measurement. Int Arch Occup Environ Health 71(2): 105-110.

Cometto-Muniz JE, Cain WS and Abraham MH (1998). Nasal pungency and odor of homologous aldehydes and carboxylic acids. Exp Brain Res 118(2): 180-188.

Cometto-Muniz JE, Cain WS, Abraham MH and Gola JM (1999). Chemosensory detectability of 1-butanol and 2-heptanone singly and in binary mixtures. Physiol Behav 67(2): 269-276.

Cometto-Muniz JE, Cain WS, Abraham MH and Gola JM (2001). Ocular and nasal trigeminal detection of butyl acetate and toluene presented singly and in mixtures. Toxicol Sci 63(2): 233-244.

Cometto-Muniz JE, Cain WS, Abraham MH and Gola JM (2002). Psychometric functions for the olfactory and trigeminal detectability of butyl acetate and toluene. J Appl Toxicol 22(1): 25-30.

Delfino RJ, Gong H, Jr., Linn WS, Pellizzari ED and Hu Y (2003). Asthma symptoms in hispanic children and daily ambient exposures to toxic and criteria air pollutants. Environ Health Perspect 111(4): 647-656.

Dellarco VL (1988). A mutagenicity assessment of acetaldehyde. Mutat Res 195(1): 1-20.

Donohue Jr. TM, Tuma DJ and Sorrell MF (1983). Acetaldehyde adducts with proteins: Binding of [^{14}C]acetaldehyde to serum albumin. Arch Biochem Biophys 220(1): 239-246.

Dorman DC, Struve MF, Wong BA, Gross EA, Parkinson C, Willson GA, Tan YM, Campbell JL, Teeguarden JG, Clewell HJ, 3rd and Andersen ME (2008). Derivation of an inhalation reference concentration based upon olfactory neuronal loss in male rats following subchronic acetaldehyde inhalation. Inhal Toxicol 20(3): 245-56.

Doty RL, Cometto-Muniz JE, Jalowayski AA, Dalton P, Kendal-Reed M and Hodgson M (2004). Assessment of upper respiratory tract and ocular irritative effects of volatile chemicals in humans. Crit Rev Toxicol 34(2): 85-142.

Egle Jr JL (1970). Retention of inhaled acetaldehyde in man. J Pharmacol Exp Ther 174(1): 14-9.

Egle Jr. JL (1972a). Effects of inhaled acetaldehyde and propionaldehyde on blood pressure and heart rate. Toxicol Appl Pharm 23(1): 131-135.

Egle Jr. JL (1972b). Retention of inhaled acetaldehyde in the dog. Arch Environ Health 24(5): 354-357.

Eriksson CJ (2001). The role of acetaldehyde in the actions of alcohol (update 2000). Alcohol Clin Exp Res 25(5 Suppl ISBRA): 15S-32S.

Eriksson CJ, Sippel HW and Forsander OA (1977). The occurrence of acetaldehyde binding in rat blood but not in human blood. FEBS Lett 75(1): 205-208.

Fenske JD and Paulson SE (1999). Human breath emissions of VOCs. J Air Waste Manag Assoc 49(5): 594-598.

Feron VJ, Arts JH, Kuper CF, Slootweg PJ and Woutersen RA (2001). Health risks associated with inhaled nasal toxicants. Crit Rev Toxicol 31(3): 313-347.

Feron VJ, Kruysse A and Woutersen RA (1982). Respiratory tract tumours in hamsters exposed to acetaldehyde vapour alone or simultaneously to benzo(a)pyrene or diethylnitrosamine. Eur J Cancer Clin Oncol 18(1): 13-31.

Frederick CB, Bush ML, Lomax LG, Black KA, Finch L, Kimbell JS, Morgan KT, Subramaniam RP, Morris JB and Ultman JS (1998). Application of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry extrapolation of acidic vapors in the upper airways. Toxicol Appl Pharm 152(1): 211-231.

Frederick CB, Gentry PR, Bush ML, Lomax LG, Black KA, Finch L, Kimbell JS, Morgan KT, Subramaniam RP, Morris JB and Ultman JS (2001). A hybrid computational fluid dynamics and physiologically based pharmacokinetic model for comparison of predicted tissue concentrations of acrylic acid and other vapors in the rat and human nasal cavities following inhalation exposure. Inhal Toxicol 13(5): 359-376.

Fujimura M, Myou S, Kamio Y, Ishiura Y, Iwasa K, Hashimoto T and Matsuda T (1999). Increased airway responsiveness to acetaldehyde in asthmatic subjects with alcohol-induced bronchoconstriction. Eur Respir J 14(1): 19-22.

Gaines KC, Salhany JM, Tuma DJ and Sorrell MF (1977). Reaction of acetaldehyde with human erythrocyte membrane proteins. FEBS Lett 75(1): 115-119.

Hodgson AT, Beal D and McIlvaine JE (2002). Sources of formaldehyde, other aldehydes and terpenes in a new manufactured house. Indoor Air 12(4): 235-242.

Hodgson AT, Shendell DG, Fisk WJ and Apte MG (2004). Comparison of predicted and derived measures of volatile organic compounds inside four new relocatable classrooms. Indoor air 14 Suppl 8: 135-44.

Hoffmann T, Meyer RJ, Sorrell MF and Tuma DJ (1993). Reaction of acetaldehyde with proteins: Formation of stable fluorescent adducts. Alcohol Clin Exp Res 17(1): 69-74.

Hollie MC, Malone RA, Skufca RM and Nelson HS (1991). Extreme variability in aerosol output of the DeVilbiss 646 jet nebulizer. Chest 100(5): 1339-44.

HSDB (2004). Hazardous Substances Data Bank. Bethesda (MD): National Library of Medicine. htttp://toxnet.nlm.nih.gov.

Isse T, Oyama T, Matsuno K, Ogawa M, Narai-Suzuki R, Yamaguchi T, Murakami T, Kinaga T, Uchiyama I and Kawamoto T (2005). Paired acute inhalation test reveals that acetaldehyde toxicity is higher in aldehyde dehydrogenase 2 knockout mice than in wild-type mice. J Toxicol Sci 30(4): 329-337.

IWMB. (2003). *Building Material Emissions Study*. Integrated Waste Management Board, California Environmental Protection Agency. http://www.ciwmb.ca.gov/Publications.

Jones AW (1995). Measuring and reporting the concentration of acetaldehyde in human breath. Alcohol Alcohol 30(3): 271-285.

Kinney PL, Chillrud SN, Ramstrom S, Ross J and Spengler JD (2002). Exposures to multiple air toxics in New York City. Environ Health Perspect 110 (Suppl 4): 539-546.

Kruysse A, Feron VJ and Til HP (1975). Repeated exposure to acetaldehyde vapor. Studies in Syrian golden hamsters. Arch Environ Health 30(9): 449-452.

Lacroix G, Tissot S, Rogerieux F, Beaulieu R, Cornu L, Gillet C, Robidel F, Lefevre JP and Bois FY (2002). Decrease in ovalbumin-induced pulmonary allergic response by benzaldehyde but not acetaldehyde exposure in a guinea pig model. J Toxicol Env Heal A 65(14): 995-1012.

Lee RD, An SM, Kim SS, Rhee GS, Kwack SJ, Seok JH, Chae SY, Park CH, Choi YW, Kim HS, Cho HY, Lee BM and Park KL (2005). Neurotoxic effects of alcohol and acetaldehyde during embryonic development. J Toxicol Env Heal A 68(23-24): 2147-2162.

Lindstrom AB, Proffitt D and Fortune CR (1995). Effects of modified residential construction on indoor air quality. Indoor Air 5: 258-269.

Mannino DM, Homa DM, Pertowski CA, Ashizawa A, Nixon LL, Johnson CA, Ball LB, Jack E and Kang DS (1998). Surveillance for asthma--United States, 1960-1995. MMWR CDC Surveill Summ 47(1): 1-27.

Miller SL, Branoff S and Nazaroff WW (1998). Exposure to toxic air contaminants in environmental tobacco smoke: an assessment for California based on personal monitoring data. J Expo Anal Env Epid 8(3): 287-311.

Mohammad A, Olcott HS and Fraenkel-Conrat H (1949). The reaction of proteins with acetaldehyde. Arch Biochem 24(2): 270-280.

Morris JB (1997a). Dosimetry, toxicity and carcinogenicity of inspired acetaldehyde in the rat. Mutat Res 380(1-2): 113-124.

Morris JB (1997b). Uptake of acetaldehyde vapor and aldehyde dehydrogenase levels in the upper respiratory tracts of the mouse, rat, hamster and guinea pig. Fundam Appl Toxicol 35(1): 91-100.

Morris JB and Blanchard KT (1992). Upper respiratory tract deposition of inspired acetaldehyde. Toxicol Appl Pharm 114(1): 140-146.

Myou S, Fujimura M, Nishi K, Matsuda M, Ohka T and Matsuda T (1994). Potentiating effect of inhaled acetaldehyde on bronchial responsiveness to methacholine in asthmatic subjects. Thorax 49(7): 644-648.

Myou S, Fujimura M, Nishi K, Ohka T and Matsuda T (1993). Aerosolized acetaldehyde induces histamine-mediated bronchoconstriction in asthmatics. Am Rev Respir Dis 148(4 Pt 1): 940-943.

Myou S, Fujimura M, Kamio Y, Bando T, Nakatsumi Y and Matsuda T (1995). Repeated inhalation challenge with exogenous and endogenous histamine released by acetaldehyde inhalation in asthmatic patients. Am J Respir Crit Care Med 152(2): 456-460.

NAS. (2004). *Acute Exposure Guideline Levels (AEGLs) for Acetaldehyde* CAS Reg. No. 75-07-0. Washington D.C.: National Advisory Committee/AEGL, U.S. Environmental Protection Agency.

Nazaroff WW (2004). Inhalation of hazardous air pollutants from environmental tobacco smoke in US residences. J Expo Anal Env Epid 14: S71-S77.

OEHHA. (2001). *Prioritization of Toxic Air Contaminants - Children's Health Protection Act - Final Report*. Sacramento (CA): Air Toxics and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/air/toxic_contaminants/SB25finalreport.html.

OEHHA. (2006). *Safe Drinking Water and Toxic Enforcement Act of 1986. Chemicals Known to the State to Cause Cancer or Reproductive Toxicity. August 11, 2006*. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/prop65/prop65_list/files/P65single081106.pdf.

Plowchalk DR, Andersen ME and Bogdanffy MS (1997). Physiologically based modeling of vinyl acetate uptake, metabolism, and intracellular pH changes in the rat nasal cavity. Toxicol Appl Pharmacol 142(2): 386-400.

Pratt OE (1980). The fetal alcohol syndrome: Transport of nutrients and transfer of alcohol and acetaldehyde from mother to fetus. Psychopharmacol Alcohol: 229-256.

Prieto L. (2008) Personal communication with author regarding 95% confidence interval on data from Prieto et al., 2000. Communication via email received August 21, 2008 and November 20, 2008.

Prieto L, Gutierrez V, Cervera A and Linana J (2002a). Airway obstruction induced by inhaled acetaldehyde in asthma: Repeatability and relationship to adenosine 5'-monophosphate responsiveness. J Invest Allerg Clin Immuno 12(2): 91-98.

Prieto L, Sanchez-Toril F, Brotons B, Soriano S, Casan R and Belenguer JL (2000). Airway responsiveness to acetaldehyde in patients with asthma: Relationship to methacholine responsiveness and peak expiratory flow variation. Clin Exp Allergy 30(1): 71-78.

Prieto L, Sanchez-Toril F, Gutierrez V and Marin MJ (2002b). Airway responsiveness to inhaled acetaldehyde in subjects with allergic rhinitis: Relationship to methacholine responsiveness. Respiration 69(2): 129-135.

Saito Y, Sasaki F, Tanaka I, Sato M, Okazawa M, Sakakibara H and Suetsugu S (2001). Acute severe alcohol-induced bronchial asthma. Intern Med 40(7): 643-645.

Saldiva PH, do Rio Caldeira MP, Massad E, Calheiros DF, Cardoso LM, Bohm GM and Saldiva CD (1985). Effects of formaldehyde and acetaldehyde inhalation on rat pulmonary mechanics. J Appl Toxicol 5(5): 288-292.

Shaskan EG and Dolinsky ZS (1985). Elevated endogenous breath acetaldehyde levels among abusers of alcohol and cigarettes. Prog Neuro-Psychoph 9(3): 267-272.

Shiohara E, Tsukada M, Chiba S, Yamazaki H, Nishiguchi K, Miyamoto R and Nakanishi S (1984). Subcellular aldehyde dehydrogenase activity and acetaldehyde oxidation by isolated intact mitochondria of rat brain and liver after acetaldehyde treatment. Toxicology 30(1): 25-30.

Silverman L, Schultes HF and First MW (1946). Further studies on sensory response to certain industrial solvent vapors. J Ind Hyg Toxicol 28: 262-266.

Skog E (1950). A toxicological investigation of lower aliphatic aldehydes. I. Toxicity of formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, as well as acrolein and crotonaldehyde. Acta Pharmacol Toxicol 6: 299-318.

Sreenathan RN, Padmanabhan R and Singh S (1982). Teratogenic effects of acetaldehyde in the rat. Drug Alcohol Depend 9(4): 339-350.

Stanek JJ and Morris JB (1999). The effect of inhibition of aldehyde dehydrogenase on nasal uptake of inspired acetaldehyde. Toxicol Sci 49(2): 225-231.

Steinhagen WH and Barrow CS (1984). Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice. Toxicol Appl Pharm 72(3): 495-503.

Tardif R, Liu L and Raizenne M (2004). Exhaled ethanol and acetaldehyde in human subjects exposed to low levels of ethanol. Inhal Toxicol 16(4): 203-207.

Teeguarden JG, Bogdanffy MS, Covington TR, Tan C and Jarabek AM (2008). A PBPK model for evaluating the impact of aldehyde dehydrogenase polymorphisms on comparative rat and human nasal tissue acetaldehyde dosimetry. Inhal Toxicol 20(4): 375-90.

Thompson PA and Folb PI (1982). An in vitro model of alcohol and acetaldehyde teratogenicity. J Appl Toxicol 2(4): 190-195.

Tuma DJ and Sorrell MF (1985). Covalent binding of acetaldehyde to hepatic proteins: Role in alcoholic liver injury. Prog Clin Biol Res 183: 3-17.

Tyulina OV, Prokopieva VD, Boldyrev AA and Johnson P (2006). Erythrocyte and plasma protein modification in alcoholism: A possible role of acetaldehyde. Biochim Biophys Acta-Molecular Basis of Disease 1762(5): 558-563.

U.S. EPA. (2003). Benchmark Dose Software. National Center for Environmental Assessment, United States Environmental Protection Agency.

Watanabe A, Hobara N and Nagashima H (1986). Blood and liver acetaldehyde concentration in rats following acetaldehyde inhalation and intravenous and intragastric ethanol administration. Bull Environ Contam Toxicol 37(4): 513-516.

West JR (1994). Recent findings on the mechanisms by which alcohol damages the developing nervous system. Alcohol Clin Exp Res 18(2): 9A.

Wickramasinghe SN, Thomas S and Hasan R (1994). Reaction of ^{14}C-acetaldehyde with whole blood in vitro: Further evidence for the formation of unstable complexes with plasma proteins and red cells. Alcohol Alcohol 29(1): 51-57.

Woutersen RA, Appelman LM, Feron VJ and Van der Heijden CA (1984). Inhalation toxicity of acetaldehyde in rats. II. Carcinogenicity study: Interim results after 15 months. Toxicology 31(2): 123-133.

Woutersen RA, Appelman LM, Van Garderen-Hoetmer A and Feron VJ (1986). Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. Toxicology 41(2): 213-231.

Woutersen RA and Feron VJ (1987). Inhalation toxicity of acetaldehyde in rats. IV. Progression and regression of nasal lesions after discontinuation of exposure. Toxicology 47(3): 295-305.

Yang X, Zhang YP, Chen D, Chen WG and Wang R (2001). Eye irritation caused by formaldehyde as an indoor air pollution--a controlled human exposure experiment. Biomed Environ Sci 14(3): 229-36.

Zorzano A and Herrera E (1989a). Decreased in vivo rate of ethanol metabolism in the suckling rat. Alcohol Clin Exp Res 13(4): 527-532.

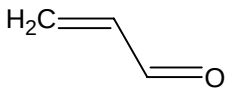
Zorzano A and Herrera E (1989b). Disposition of ethanol and acetaldehyde in late pregnant rats and their fetuses. Pediatr Res 25(1): 102-106.

Zweidinger R, Hoffman A and Gage L (1990). Comparison of Indoor and Outdoor Aldehyde Concentrations During the IACP Roanoke, Virginia USA Residential Study. Measurement of Toxic and Related Air Pollutants International Symposium.Atmospheric Research and Exposure Assessment Laboratory and Air and Waste Mangement Association, United States Environmental Protection Agency. pp 994-999. Raleigh (NC), May 1-4, 1990

Acrolein Reference Exposure Levels

(2-propenal, acrylic aldehyde, acryladehyde, acraldehyde)

CAS 107-02-8



1. Summary

Acrolein is a powerful irritant. Due to its highly reactive nature, the effects of acrolein are generally limited to the site of contact; skin, eyes and mucous membranes. Inhalation exposure to low levels (≤ 1 ppm) causes irritation of the eyes, nose and throat. Acute exposures to levels above 1 ppm result in mucous hypersecretion and exacerbation of allergic airway response in animal models. Moderately higher exposures may result in severe lacrimation, and irritation of the mucous membranes of the respiratory tract. Death due to respiratory failure has been associated with high level exposures. Long term exposure to acrolein may result in structural and functional changes in the respiratory tract, including lesions in the nasal mucosa, and pulmonary inflammation. The studies reviewed for this document include those published through Spring, 2008.

1.1 Acrolein Acute REL

Reference Exposure Level	**2.5 μg/m³ (1.1 ppb)**
Critical effect(s)	Subjective ocular irritation
Hazard Index target(s)	Eyes

1.2 Acrolein 8-Hour REL

Reference Exposure Level	**0.70 μg/m³ (0.30 ppb)**
Critical effect(s)	Lesions in respiratory epithelium
Hazard Index target(s)	Respiratory

1.3 Acrolein Chronic REL

Reference Exposure Level	**0.35 μg/m³ (0.15 ppb)**
Critical effect(s)	Lesions in respiratory epithelium
Hazard Index target(s)	Respiratory

2. Physical & Chemical Properties

Description	Colorless or yellow liquid with piercing disagreeable odor
Molecular formula	C_3H_4O
Molecular weight	56.1 g/mol
Density	0.843 g/cm^3 @ 20° C
Boiling point	53° C
Melting point	-87° C
Vapor pressure	220 mm Hg @ 20° C
Flashpoint	-26° C
Explosive limits	2.8% - 31% by volume
Solubility	soluble in ethanol, diethyl ether, and up to 20% w/v in water
Odor threshold	0.5 ppm
Metabolites	glycidaldehyde, acrylic acid
Conversion factor	1 ppm in air = 2.3 mg/m^3 @ 25° C

3. Occurrence and Major Uses

Acrolein is principally used as a chemical intermediate in the production of acrylic acid and its esters. Acrolein is used directly as an aquatic herbicide and algicide in irrigation canals, as a microbiocide in oil wells, liquid hydrocarbon fuels, cooling-water towers and water-treatment ponds, and as a slimicide in the manufacture of paper (IARC, 1995). Combustion of fossil fuels, tobacco smoke, and pyrolyzed animal and vegetable fats contribute to the environmental prevalence of acrolein. Acrolein is a byproduct of fires and is one of several acute toxicants to which firefighters are exposed. It is also formed from atmospheric reactions of 1,3-butadiene. The annual statewide emissions of acrolein from mobile, stationary and natural sources (not including atmospheric transformation) reported in the California Toxics Inventory for 2004 were estimated to be 2,242 tons contributing to a statewide ambient level of 0.53 ppb (CARB, 2005b).

4. Metabolism

The metabolism of acrolein comprises several pathways. It rapidly reacts with sulfhydryl groups, especially protein cysteine residues and glutathione. The glutathione conjugate may be oxidized or reduced to mercapturic acids (N-acetyl –S-2-carboxyethylcysteine and N-acetyl-S-3-hydroxypropylcysteine, respectively), with the reduction pathway predominating, followed by urinary elimination. Alternatively, acrolein may be epoxidized to glycidaldehyde, which is in turn attacked by glutathione and oxidatively processed to the mercapturic acid, N-acetyl-S-2-carboxy-2-hydroxyethylcysteine. In a third pathway, the Michael addition of water to acrolein is followed by oxidation to malonic and finally oxalic acids (Parent et al., 1998). The formation of homopolymers of acrolein is thought to occur but appears to be limited to the gut. Acrolein may also be oxidized to acrylic acid, mainly in the liver. Following inhalation exposure, the predominant metabolites are the 3-hydroxypropyl and 2-carboxyethyl mercapturic acids mentioned above (Linhart et al., 1996).

5. Acute Toxicity of Acrolein

5.1 Acute Toxicity to Adult Humans

Sensory irritation is the primary adverse effect associated with acute, low level exposures to acrolein. The irritative effects of acrolein are noticeable at low levels of exposure (≤0.25 ppm) and rapidly become more pronounced with increasing concentration; brief exposure (1.5 min) to 0.3 ppm (0.7 mg/m³) causes irritation of the eyes and nose (Weber-Tschopp et al., 1977). The powerful irritant and lacrimator properties of acrolein led to its use in gas grenades and artillery shells by the French in 1916. At a concentration of 7 mg/m³, acrolein caused severe lacrimation and irritation of the mucous membranes of the respiratory tract (Prentiss, 1937). A case report of respiratory failure and death in individuals exposed to vapors from overheated frying pans containing fat and food items implicated acrolein as the principal toxicant (Gosselin et al., 1979).

Ocular irritation is one of the most sensitive responses to acrolein. In a study by Darley et al. (1960), 36 human volunteers were exposed to 0.06, 1.3-1.6, and 2.0-2.3 ppm for 5 minutes. Acrolein was dissolved in water and delivered to the eyes in a stream of oxygen through face masks. Carbon-filter respirators were worn during exposure so that only the eyes were exposed to the test material. The subjects, who were without a history of chronic upper respiratory or eye problems, rated the degree of eye irritation every 30 seconds during exposure as none (0), medium (1), or severe (2). The individuals' maximum values were used in the analysis that revealed a concentration-dependent incidence of eye irritation (Table 5.1.1). The lowest observed adverse effect level (LOAEL) for eye irritation in human volunteers was estimated by an unspecified method to be 0.06 ppm (0.14 mg/m³) acrolein during the five minute exposures. A NOAEL was not observed in this study.

TABLE 5.1.1 OCULAR IRRITATION WITH ACROLEIN (FROM DARLEY ET AL., 1960)

Acrolein concentration	Irritation score
Filtered air	0.283
0.06 ppm	0.471
1.3-1.6 ppm	1.182
2.0-2.3 ppm	1.476

Ocular and upper respiratory tract irritation were also examined in a chamber study by Weber-Tschopp et al. (1977) involving healthy volunteers. Thirty one men and 22 women were exposed to increasing acrolein levels (0-0.60 ppm) for 40 min, while 21 men and 25 women were exposed to a constant 0.3 ppm for 60 min. Subjective reports of irritation and annoyance, and objective measures of eye-blink and respiratory rates were taken during the exposure periods. During exposure to increasing levels of acrolein, eye irritation, as measured by subjective report and blink frequency, was a more sensitive measure of irritation than nasal irritation. By comparison, for less reactive volatile compounds in studies surveyed by Doty et al. (2004), the thresholds for ocular and intranasal irritation were of the same magnitude. In the Weber-Tschopp study of acrolein, significantly ($p<0.01$) higher eye irritation was first observed at 0.07 ppm, and nasal irritation at 0.26 ppm compared to controls. Significant depression of respiratory

rates was observed at 0.60 ppm ($p<0.05$). With continuous exposure to 0.3 ppm acrolein, subjective eye and nasal irritation increased rapidly during the first 20 minutes and tended to plateau by 40 min. After 10 min of continuous exposure, a decrease in respiratory rate of 10% was evident in 47% of the subjects, while eye blink rate doubled in 66%. The authors suggest a threshold for adverse effects in the range of 0.09-0.30 ppm.

The effects of irritants such as acrolein may be accentuated in individuals with prior sensitization. Roux et al. (1999) investigated the interaction between passive sensitization of human isolated airways and exposure to pollutants (specifically, ozone and acrolein). Lung tissue from nonatopic, nonasthmatic patients was immunologically sensitized by incubation in sera from atopic asthmatic patients. Roux et al. reported that *in vitro* passive sensitization of the isolated tissues and exposure to acrolein act in a synergistic manner on human bronchial smooth muscle reactivity in response to both specific and nonspecific agonists. In tissues sensitized by incubation in sera from asthmatic patients, preexposure to 0.3 μM acrolein for 10 or 20 minutes significantly increased the maximal contractile response to a specific antigen of the dust mite, *Dermatophagoides pteronyssinus*, by 20.5 ± 6.5 % and 34.9 ± 7.4%, respectively. In addition, in sensitized tissue pre-exposed to 0.3 μM acrolein for 10 minutes, contractile response was increased by 33.5 ± 6.2% and 32.5 ± 5.1% for carbachol and histamine, respectively. Thus acrolein exposure potentially exacerbates asthma.

Mucus hypersecretion is one of the hallmarks of inflammatory airway disorders, including asthma. Borchers et al. (1999b) examined the effect of 0.01-100 nM acrolein on mucus glycoprotein (mucin) gene expression in cultured human airway epithelial cells. After a 4 hour exposure to acrolein in vitro, epithelial cells were found to have elevated mucin mRNA levels. It is not clear whether acrolein acts directly on epithelial cells or indirectly through inflammatory mediators released after acrolein exposure, however, asthma exacerbation is a likely result of acrolein exposure in susceptible individuals.

Persons with pre-existing eye, skin, respiratory, allergic, asthmatic or heart conditions might be at increased risk due to acrolein exposure. As a respiratory irritant, there is evidence that acrolein exacerbates asthma via the induction of bronchial hyper-responsiveness (Leikauf et al., 1989a; Leikauf et al., 1989b; Borchers et al., 1998; Borchers et al., 1999a; Borchers et al., 1999b). Acrolein has been listed as a TAC that may disproportionately impact children due to concerns related to asthma exacerbation.

Cancer patients treated with cyclophosphamide could be at increased risk because acrolein is a metabolite of cyclophosphamide (NTIS, 1981).

The effects of acrolein as an ocular irritant may be enhanced among those who wear contact lenses. Although no data specific to acrolein in this context were located, observations of ocular irritation following exposure to formaldehyde in an anatomy dissecting laboratory may be germane. Tanaka et al. (2003) reported that formaldehyde levels in an anatomy lab peaked at 0.62 ppm shortly after the exposure of cadavers for dissection, with a gradual decrease to 0.11 ppm. Formaldehyde-related irritation of the eyes, nose, throat, airways and skin was reported by 59% of the students. Ocular irritation was significantly ($p < 0.001$) higher among wearers of contact lenses compared with students without contacts. The ability of contact lenses to trap and concentrate volatile compounds, and to extend the exposure time by limiting the eye's normal

self-cleansing, may make contact lens wearers more susceptible to ocular exposure and irritation by acrolein.

5.2 Acute Toxicity to Infants and Children

The literature specifically examining the effects of acrolein inhalation in infants and children is limited and comprises case studies of accidental exposure, and exposures to multiple substances. The most frequent sources of acrolein in childhood exposures are environmental tobacco smoke (ETS) and acrolein formed from overheated cooking oils. Mahut et al. (1993) describe the case of a 27 month-old boy hospitalized for acute respiratory failure following exposure for about an hour to acrid smoke from vegetable oil burning on an electric hot plate. The child was reportedly cyanotic with labored, crackling breathing, and was experiencing severe respiratory acidosis. Eighteen months following exposure, X-ray and CT scans showed bronchial thickening, massive over-inflation, patchy emphysema and diffuse bronchiectasis. In this case, and in cases of exposure to ETS, infants may be more susceptible to the adverse effects of acrolein in part due to an inability to escape exposure. Children also may be more susceptible to the effects of respiratory irritants due to the immature state of their airways.

As noted in OEHHA (2001): "*OEHHA considers asthma to impact children more than adults. Children have higher prevalence rates of asthma than do adults (Mannino et al., 1998). In addition, asthma episodes can be more severe due to the smaller airways of children, and result in more hospitalizations in children, particularly from the ages of 0 to 4 years, than in adults (Mannino et al., 1998; CDHS, 2000)." "Thus, on a population-wide basis, children are more impacted by asthma than adults, and since acrolein exacerbates asthma, children may be more impacted by acrolein toxicity than adults."* Data strongly suggesting that acrolein exacerbates asthma derive from studies using human tissue *in vitro* (Roux et al., 1999; Borchers et al., 1999a) and in animals *in vivo* (Leikauf et al., 1989a; 1989b; Borchers et al., 1998; Borchers et al., 1999b).

5.3 Acute Toxicity to Experimental Animals

Experimental exposures of rodents to acrolein at and above levels that are irritating to the eyes and respiratory tract in humans provide evidence for several adverse effects and their possible mechanisms. Acrolein prompts a proliferative response in nasal epithelium as shown by increased DNA synthesis (Roemer et al., 1993) and expression of mucin genes (Borchers et al., 1998). The latter effect in turn is associated with the hyper-secretion of mucus that may contribute to chronic obstructive pulmonary disease and asthma (Borchers et al., 1998). Bronchial hyper-responsiveness, a hallmark of asthma, increases with acrolein exposure (Leikauf et al., 1989a) supporting a connection between acrolein exposure and exacerbation of asthma in humans. The dose-dependent decreases in protective epithelial enzyme activities (Cassee et al., 1996b) and levels of sulfhydryls (Lam et al., 1985; McNulty et al., 1984) are likely to be involved in the observed formation of lesions in the nasal epithelium (Cassee et al., 1996b).

TABLE 5.3.1 ACROLEIN EFFECTS IN EXPERIMENTAL ANIMALS

Study	Model	Exposure	Outcome
Roemer et al. 1993	Proliferation of rat nasal and tracheal epithelium	0, 0.2, 0.6 ppm 6 h/d, 1 or 3 d	Increased DNA synthesis at 0.2 ppm (LOAEL)
Borchers et al. 1998	Mucus hyper-secretion, mucin gene expression in rat trachea and lungs	0.3, 0.75, 1.5, 3.0 ppm 6 h/d, 5 d/w	Hyper-secretion and gene expression at 0.75 ppm. (NOAEL = 0.3 ppm)
Leikauf et al. 1989a	Bronchial hyper-responsiveness and airway resistance in guinea pigs	1.3 ppm, 2 h	Resistance increased from 0.86 to 1.29 ml·cm H_2O/ml. Acetylcholine to double airway resistance dropped from 114 to 44.7 µg/kg/min
Buckley et al. 1984	Nasal histopathology at (RD_{50}) in mice;	1.7 ppm, 6 h/d, 5d	Exfoliation and squamous metaplasia of epithelium
Morris et al. 2003	Decrease in respiratory rate (RD_{50}) in mice	0.3, 1.6, 3.9 ppm, 10 min	Control RD_{50} at 1.50 ppm vs 0.82 ppm in allergic mice
Kane et al. 1979	Decrease in respiratory rate (RD_{50}) in mice	15 min	RD_{50} 1.7 ppm
Cassee et al. 1996b	Histopathology of rat nasal epithelium	0, 0.25, 0.67, 1.4 ppm, 6 h/d, 1-3 d	Dose-dependent lesions and decreased enzyme activities in nasal epithelium
Lam et al. 1985	Sulfhydryl depletion in rat respiratory mucosa	0, 0.1, 0.5, 1.0, 2.5 ppm 3 h	Dose-dependent depletion of non-protein sulfhydryls
McNulty et al. 1984	Sulfhydryl depletion in rat respiratory mucosa and liver	0.1, 0.3, 1, 2.5, 5 ppm 3 h	Dose-dependent depletion of non-protein sulfhydryls in nasal mucosa but not liver

Roemer et al. (1993) exposed Male Sprague Dawley rats by inhalation to 0, 0.2 or 0.6 ppm acrolein for 6 hours per day on one or three successive days. Nasal and tracheal epithelial and free lung cells were analyzed for proliferative responses using 5-bromodeoxyuridine (BrdU) labeling to identify DNA synthesizing cells. A single exposure to acrolein increased the DNA synthesizing cells 3-fold. After three exposures the increase was distinctly lower. All sites analyzed showed approximately the same concentration/response pattern. Since significant changes in cell proliferation were detected at 0.2 ppm (0.46 mg/m^3) acrolein, it is a LOAEL for this experiment.

Enhanced mucus secretion is a normal airway response to inhaled irritants. However, mucus hypersecretion is involved in the development of chronic obstructive pulmonary diseases; as such, it is considered an adverse effect. Borchers et al. (1998) exposed male rats to 3.0 ppm acrolein for 6 hours/day, 5 days/week for up to 12 days and examined the lungs and trachea for mucin cell metaplasia and expression of the mucin genes MUC2 and MUC5ac. The effects of acrolein concentration on mucin mRNA levels were further examined in rats exposed daily to 0.3, 0.75, 1.5, 3.0 ppm. Acrolein exposure resulted in a time-dependent increase in mucous cell differentiation and mucus hypersecretion in rat lungs. These changes were accompanied by increases in lung MUC5ac mRNA to levels 3-fold higher than in controls, and readily immunohistochemically detectable levels of MUC5ac. MUC5ac mRNA was elevated by concentrations as low as 0.75 ppm while MUC2 mRNA was not affected by any of the levels tested. Thus 0.3 ppm (0.69 mg/m^3) is a NOEL for this effect. The trachea of treated animals showed sloughing of the epithelium accompanied by excessive mucus and inflammatory cells in the lumen.

Bronchial hyper-responsiveness is a hallmark of reactive airway diseases such as asthma, and may be induced by inhaled irritants. Leikauf et al. (1989a) exposed guinea pigs to 1.3 ppm acrolein for 2 hours and measured the induction of bronchial hyperresponsiveness by the amount of infused acetylcholine necessary to double specific airway resistance 1, 2, 6, and 24 hours after exposure compared to baseline. The dose of acetylcholine required to double airway resistance decreased from 114.0 ± 6.6 to 44.7 ± 4.2 µg/kg/min ($p < 0.001$) at 2 hours following acrolein exposure and remained low for at least 24 hours. Acrolein exposure was found to increase levels of the bronchoconstrictor leukotriene C_4 (LTC_4) in bronchoalveolar lavage fluids prior to the observation of bronchial hyperresponsiveness. This hyperresponsiveness was prevented by treatment with an inhibitor of LTC_4 synthesis or an LTC_4 receptor antagonist. Acrolein was thus shown to induce bronchial hyperresponsiveness, an effect apparently mediated by LTC_4.

Buckley et al. (1984) investigated whether lesions occur in the respiratory tract of Swiss-Webster mice after exposure to the RD_{50} concentrations of ten sensory irritants including acrolein. After exposure of mice for 6 hr/day for 5 days to 1.7 ppm acrolein, the respiratory tract was examined for histopathologic changes. Acrolein (and all other irritants) produced lesions in the nasal cavity with a distinct anterior-posterior severity gradient. Acrolein specifically caused severe exfoliation and squamous metaplasia of the respiratory epithelium and moderate ulceration of the olfactory epithelium. Acrolein did not induce lesions in the lower respiratory tract.

Morris et al. (2003) compared the respiratory responses to acrolein in healthy mice with those in mice previously sensitized to ovalbumin. Inhalation exposure to ovalbumin prior to acrolein

exposure elicited an allergic response in the sensitized mice that was characteristic of allergic airway disease. Upon subsequent acrolein exposure, the RD_{50}, a measure of the dose required to reduce the respiratory rate by 50%, was 1.50 ppm in naïve mice and 0.82 ppm in the mouse model of allergic airway disease. Thus in sensitized animals, a lower concentration of acrolein is required to elicit the same changes in breathing rate observed in non-allergic animals. In both intact mice and in isolated mouse upper respiratory tracts, acrolein exposure caused a significant ($P < 0.05$) increase in flow resistance, an effect that was immediate and not exposure time dependent. Pretreatment with capsaicin to defunctionalize sensory neurons significantly attenuated the breathing rate and obstructive responses supporting the role of sensory neuron stimulation in the response to acrolein. For comparison, Kane et al. (1979) also used the RD_{50} as a measure of sensory irritation and estimated an RD_{50} of 1.7 ppm in mice during 15 minutes of acrolein exposure.

Cassee et al. (1996b) exposed male Wistar rats to 0, 0.25, 0.67, or 1.4 ppm acrolein for 6 hours per day on one or three successive days. Immediately following the last exposure, the rats were killed. Mucosae from the respiratory or olfactory parts of the nose were collected from 3 rats per group for biochemical analyses. The skulls of the other rats in each group were prepared for histopathology and cell proliferation measurements. Nasal epithelium, examined microscopically, showed dose-dependent evidence of disarrangement, necrosis, thickening, and desquamation of the respiratory/transitional epithelium (Table 5.3.2). Significant basal cell hyperplasia, observed at the lowest dose (0.25 ppm), increased with exposure. The activity of glutathione reductase (GR) was reduced after one-day exposure to acrolein, while the activities of GR, glutathione-S-transferase and aldehyde dehydrogenase were reduced following the three-day exposures. These results and those mentioned below suggest that acrolein interferes with enzyme systems involved in its detoxification.

TABLE 5.3.2 NASAL LESIONS IN RATS WITH ACROLEIN EXPOSURE

(from Cassee et al., 1996b)

Site and type of lesion	Extent	Incidence	
		Low	Medium
Noses examined		5	6
Disarrangement, necrosis, desquamation of respiratory, transitional epithelium	Slight (mainly disarrangement)	4	3
	Moderate	1	3
	Severe and extensive	0	0
Basal cell hyperplasia and/or increased mitotic figures	Slight (focal)	3	2
	Moderate	0	4
	Severe (extensive)	0	0

Pronounced and possibly irreversible biochemical changes occur with acrolein levels that are extremely irritating. Acrolein depletes glutathione (GSH) and other free thiol groups both in vitro and in vivo (McNulty et al., 1984; Lam et al., 1985; Grafstrom et al., 1987; U.S.EPA, 2003; Yang et al., 2004). Inhalation exposure of rats to a concentration of 5 ppm (11.4 mg/m^3) for 3 hours caused irreversible depletion of non-protein sulfhydryls in the nasal mucosa (Lam et al., 1985). Under similar exposure conditions, 5 ppm (11.5 mg/m^3) for 3 hours, McNulty et al.

(1984) reported a 63% decrease in glutathione in nasal mucosal but not in liver. In addition, ^{14}C-labeled acrolein has been shown to bind irreversibly to sulfhydryl groups on cytochrome P450 in rats (Gurtoo et al., 1981). The binding of acrolein to sulfhydryl groups is localized to the area of contact (e.g., nasal membranes or lung epithelium), and is not a systemic effect (Lam et al., 1985).

The pulmonary immunological defense against a bacterial challenge using *Staphylococcus aureus* in mice was impaired in a dose-dependent manner following a single exposure to acrolein at concentrations of 3 and 6 ppm (6.9 and 13.8 mg/m^3) for 8 hours (Astry and Jakab, 1983). In this study, the control exposure was not described.

The efficiency with which acrolein enters cells of the respiratory tract in large part determines the inspired levels at which toxic effects are observed. Struve et al. (2008) measured the uptake efficiency of 0.6, 1.8 or 3.6 ppm acrolein in isolated upper respiratory tracts of anesthetized, naïve rats under constant velocity, unidirectional flow rates of 100 or 300 ml/min for up to 80 min. Similar studies were also performed on rats with previous exposure to 0.6 or 1.8 ppm acrolein for 6 hours per day, 5 days per week for 14 exposure days prior to nasal uptake studies with 1.8 or 3.6 ppm at 100 ml/min flow rate. Acrolein levels entering and exiting the isolated respiratory tract were measured to determine uptake efficiency. At the end of the exposure period, the animals were killed and the nasal respiratory and olfactory mucosa isolated for measurements of protein, and total and oxidized glutathione. The efficiency of acrolein uptake by the rat nose was dependent on the concentration, flow rate, and duration of acrolein exposure. Uptake efficiency was significantly higher at the lowest exposure than at the higher levels ($0.6 > 1.8 \approx 3.6$; $p < 0.0001$), and at the lower flow rate ($100 > 300$; $p < 0.0061$). At both flow rates, the efficiency of uptake significantly declined over the 80 min exposure period ($p < 0.001$), with a significant interaction between concentration and time ($p = 0.01$). In naïve rats, glutathione levels dropped in respiratory epithelium but remained largely the same in olfactory epithelium. By comparison, in pre-exposed rats, the acrolein uptake efficiency was higher than in naïve rats. However, the GSH levels at the end of exposure were also higher, perhaps suggesting an adaptive response.

6. Chronic Toxicity of Acrolein

6.1 Chronic Toxicity to Adult Humans

Information regarding the chronic toxicity of acrolein in humans is limited. There is inadequate direct evidence for carcinogenicity of acrolein in humans or experimental animals. However, a metabolite of acrolein, the reactive epoxide glycidaldehyde, has been shown to be mutagenic and carcinogenic in mice and rats (IARC, 1985). Therefore, acrolein has been designated a Group C substance, with possible human carcinogenic potential by the U.S.EPA (1987). In addition, acrolein-DNA adducts have been found in aortic tissue following 6 hour inhalation exposure to 1 and 10 ppm acrolein (Penn et al., 2001).

A source of chronic acrolein exposure for some individuals is tobacco smoking. Much of the pulmonary irritancy associated with tobacco smoke has been attributed to acrolein and research in this area suggests mechanisms for some of acrolein's pulmonary effects. As part of a defense response, pulmonary neutrophils release oxidants, proteases and cytokines such as IL-8, all of

which may promote inflammation and potentiate tissue damage. To limit tissue damage and resolve the inflammation, neutrophils normally undergo constitutive apoptosis. Experiments with isolated human neutrophils exposed to acrolein at levels achievable during active smoking (1-50 µM) found that acrolein inhibited neutrophil apoptosis, increased IL-8 production, and activated mitogen-activated protein kinases (MAPK) (Finkelstein et al., 2001). At acrolein concentrations up to 10 µM, inhibition of apoptosis was accompanied by increased cell viability. At higher acrolein levels, cell viability decreased as necrotic cell death increased. While the mechanisms behind acrolein's concentration-dependent effects on neutrophils are not clear, the effects observed at the lower exposure levels suggest that acrolein may contribute to pulmonary inflammation and exacerbate allergic responses by prolonging the survival of neutrophils, and stimulating the production of inflammation-related cytokines and enzymes. At higher levels, frank cellular toxicity becomes more prominent.

6.2 Chronic Toxicity to Infants and Children

No data addressing the effects of chronic acrolein exposure among infants and children were located. Inasmuch as acrolein is one of the major irritants in environmental tobacco smoke (Takabe et al., 2001) at relatively high concentrations in smokers' homes (1.6-3.6 µg/m^3; 0.70-1.57 ppb (Nazaroff and Singer, 2004)), children living with smokers may be disproportionately exposed to acrolein as they are less able to avoid exposure than are adult nonsmokers. To the extent that respiratory irritants such as acrolein elicit bronchoconstriction and excessive mucus secretion characteristic of asthma, children, with their smaller airways and greater prevalence of asthma, may experience more diminution of pulmonary function and more episodes of asthma with chronic exposure.

6.3 Chronic Toxicity to Experimental Animals

Long-term exposure to acrolein causes structural and functional changes in the respiratory tract. Nasal and pulmonary effects following acrolein exposure for 13 weeks (6 hours/day, 5 days/week) were described by Dorman et al. (2008) in 360 male F344 rats. The whole-body exposures were to air concentrations of 0, 0.02, 0.06, 0.2, 0.6, and 1.8 ppm acrolein, with evaluation of respiratory tract histopathology after 4, 14, 30 and 65 days of exposure, and at 60 days following the end of the 13 week exposure. Body weights of all acrolein exposed rats were depressed but there were reportedly no other significant increases in clinical signs. Formalin-fixed noses were sectioned transversely providing six sections of the nasal cavity at standard levels. Larynx, trachea and lungs were fixed, stained with hematoxylin and eosin, and examined histologically. The study examined both respiratory and olfactory epithelia with the former being the more sensitive as evidenced by inflammation, hyperplasia and squamous metaplasia. Mild hyperplasia of the respiratory epithelia was first observed after 4 days of exposure to $\geq$ 0.6 ppm. The NOAEL for pathology of nasal respiratory epithelia was 0.2 ppm in the lateral walls of level II, and for olfactory epithelia, 0.6 ppm. At the highest concentration, 1.8 ppm, mild squamous metaplasia was also observed in the larynx and trachea, but no treatment related effects were seen in the lungs. Two months following cessation of exposure, only partial recovery of the olfactory epithelium was observed; primarily in caudal areas where lesions developed more slowly and were less severe.

Schroeter et al. (2008) used data from the above study by Dorman et al. (2008) for the development of a physiological computational fluid dynamics (CFD) model of acrolein nasal dosimetry. The CFD models of Kimbell et al. (1997) and Subramaniam et al. (1998) were modified to estimate kinetic parameters of acrolein flux in rat nasal passages, and allow a cross-species prediction of acrolein flux in humans associated with histopathology. Based on a NOAEL of 0.6 ppm and a LOAEL of 1.8 ppm for olfactory neuronal loss from Dorman et al. (2008), the CFD model predicted a threshold acrolein flux of 72 pg/cm^2-s at region 11, comprising portions of the third ethmoturbinate. Assuming equal tissue doses of acrolein elicit similar responses in the olfactory epithelium of rats and human, an exposure level that may be expected to represent the threshold for olfactory neuronal loss in humans may be estimated. The 99th percentile olfactory flux value that is equal to the threshold of 72 pg/cm^2-s was estimated to be 45 ppb. The authors use this concentration to estimate a human equivalent NOAEL of 8 ppb, and a reference concentration (RfC) of 0.27 ppb. However, the threshold acrolein flux associated with the lower NOAEL of 0.2 ppm, reported by Dorman et al. (2008) for respiratory epithelium, was not estimated, and an equivalent human threshold and NOAEL is not available. The rationale for this, presented in Dorman et al. (2008), is *"Our CFD modeling efforts have revealed that although the observed NOAEL for the respiratory epithelium is lower than that seen for the olfactory epithelium (i.e., 0.2 vs. 0.6 ppm), in actuality the olfactory epithelial lesion arises at an appreciably lower delivered tissue dose suggesting that the olfactory epithelium is more sensitive to the effects of inhaled acrolein than is the respiratory epithelium (Schroeter et al. (2008)."* The RfC of 0.27 ppb estimated by the authors is thus based on lesion formation at the lowest modeled tissue dose rather than on the more relevant value of the lowest applied acrolein concentration associated with an adverse effect.

Structural and functional changes in the respiratory tract were also examined in male Fischer-344 rats exposed for 6 hours/day, 5 days/week for 62 days to acrolein at concentrations of 0, 0.4, 1.4, and 4.0 ppm (0, 0.92, 3.2, and 9.2 mg/m^3)(Kutzman, 1981; Kutzman et al., 1985). Each group of 24 animals was assessed for pulmonary function immediately prior to the end of the experiment. Pulmonary function tests included lung volumes, forced respiratory capacity, pulmonary resistance, dynamic compliance, diffusing capacity of carbon monoxide, and multi-breath nitrogen washout. At the end of the experiment, animals were killed and histopathological changes in the lungs were recorded. Eight additional rats were designated for histopathology and 8 rats were used for reproductive testing only. All analyses were performed at 6 days post-exposure to minimize the acute effects of acrolein. Mortality was high (56%) in rats exposed to 4.0 ppm (9.2 mg/m^3). The observed mortality was due to acute bronchopneumonia in these cases. The animals from this group that survived had reduced body weight. No histological changes were observed in extra-respiratory tissues in any group. There was a concentration-dependent increase in histological changes to the nasal turbinates (increased submucosal lymphoid aggregates), beginning at 0.4 ppm. Concentration-dependent damage to the peribronchiolar and bronchiolar regions included epithelial necrosis and sloughed cells lying free in the lumen. No lung lesions were observed in the 0.4 ppm group. The LOAEL for nasal lesions (squamous epithelial metaplasia and neutrophil infiltration) in this study was 0.4 ppm.

Feron et al. (1978) exposed groups of 20 Syrian golden hamsters, 12 SPF Wistar rats and 4 Dutch rabbits (of both sexes) to acrolein vapor at 0, 0.4, 1.4 and 4.9 ppm (0, 0.92, 3.2, and 11.3 mg/m^3) 6 hours/day, 5 days/week for 13 weeks. The most prominent effects at the highest level included mortality in rats (3 of each sex), and ocular and nasal irritation, growth depression, and

histopathological changes of the respiratory tract in each species. The changes in the airways induced by acrolein consisted of destruction, and hyperplasia and metaplasia of the lining epithelium accompanied by inflammatory alterations. Rats were the most susceptible species examined and showed treatment-related histopathological abnormalities in the nasal cavity down to 0.4 ppm (LOAEL), whereas this level was a NOAEL in hamsters and rabbits. The results for individual rats at 0.4 ppm were not given.

Bouley et al. (1975; 1976) exposed male SPF OFA rats continuously to 0.55 ppm (1.3 mg/m^3) of acrolein for up to 63 days. This level of acrolein led to a greater susceptibility to airborne *Salmonella enteritidis* infection during the first three weeks compared to control rats but it disappeared spontaneously when exposure was continued beyond three weeks. The general toxic effect of diminished weight gain (due to reduced feeding) compared to the control group lasted as long as exposure and disappeared only after acrolein was discontinued. Sneezing, a sign of nasal irritation, was consistently observed in the exposed animals on days 7 through 21 but ceased thereafter. No histopathology of the nasal cavity of or any other tissue was reported.

In one of the few chronic studies reported, Feron and Kruysse (1977) exposed hamsters (18/gender) to 4 ppm (9.2 mg/m^3) acrolein for 7 hours/day, 5 days/week, for 52 weeks. Mild to moderate histological changes were observed in the upper and lower respiratory tract. No evidence of toxicity to other organs was apparent at necropsy, although body weight was decreased. Hematology, urinalysis, and serum enzymes were not affected by exposure. Thus 4 ppm is a chronic LOAEL for hamsters. As noted above, hamsters appear to be a less sensitive species than rats (Feron et al., 1978).

Exposures of rodents have generally formed the basis for the determination of acrolein's chronic effects. However, an interspecies comparison was conducted by Lyon and associates (Lyon et al., 1970) who investigated the effects of repeated or continuous exposures of acrolein on Sprague-Dawley rats (n = 15/exposure group), guinea pigs (n = 15), beagle dogs (n = 2), and male squirrel monkeys (n = 9). Animals were exposed to 0.7 or 3.7 ppm (1.6 or 8.5 mg/m^3) acrolein for 8 hours/day, 5 days/week, for 6 weeks, or continuously to 0.22, 1.0, or 1.8 ppm (0.5, 2.3, or 4.1 mg/m^3) for 90 days. The results below suggest that dogs and monkeys were more susceptible to acrolein's effects than were the rodents.

Two monkeys in the 3.7 ppm intermittent exposure group died within 9 days. Monkeys and dogs salivated excessively during the first week. Squamous metaplasia and basal cell hyperplasia of the trachea were observed in monkeys and dogs; 7 of the 9 monkeys repeatedly exposed to 3.7 ppm also exhibited bronchiolitis obliterans with squamous metaplasia in the lungs. Bronchopneumonia was noted in the dogs. Inflammation in the lung interstitiae was more prominent in the dogs than in the monkeys. Rats and guinea pigs did not exhibit signs of toxicity when exposed intermittently to 3.7 ppm. Continuous exposure to 1.0 and 1.8 ppm, but not 0.22 ppm acrolein, resulted in salivation and ocular discharge in the monkeys and dogs. Rats and guinea pigs appeared normal at all concentrations. Rats exhibited significant weight loss in the 1.0 and 1.8 ppm continuous exposure groups. Nonspecific inflammatory changes were observed in sections of brain, heart, lung, liver and kidney from all species exposed to 1.8 ppm. The lungs from the dogs showed confluent bronchopneumonia. Focal histological changes in the bronchiolar region and the spleen were detected at 0.22 ppm in dogs. Nonspecific inflammatory changes at the 0.22 ppm level were apparent in liver, lung, kidney and heart from monkeys,

guinea pigs and dogs. Unfortunately the nasal cavity was not examined in this study. While there were no unexposed control animals for any species, the cross-species comparison shows substantial interspecies variability in susceptibility.

7. Developmental and Reproductive Toxicity

There are no reports of reproductive or developmental toxicity following inhalation exposure to acrolein in humans. Kutzman (1981) studied reproductive fitness in male and female rats following acrolein inhalation for 6 hours/day, 5 days/week for 62 days. Treated males were mated with untreated females, and treated females with untreated males. No treatment-related differences were found in the parameters assessed including pregnancy rate, number of corpora lutea, embryo viability, early and late deaths, and preimplantation losses. Similarly, the morphology of sperm collected from the epididymides of treated males was examined and reportedly not affected. Bouley et al. (1975; 1976) exposed three male and 21 female SPF-OFA rats continuously to 0.55 ppm (1.26 mg/m^3) acrolein vapor for 25 days. The rats were allowed to mate on day 4 of the exposure. The number of acrolein-exposed pregnant rats and the number and mean body weight of their fetuses were similar to controls.

In rats, acrolein can induce teratogenic and embryotoxic effects when administered directly into the amniotic fluid, or when added to cultured rat embryos (Slott and Hales, 1986). Additionally, acrolein injected into chicken embryos resulted in embryotoxicity and some teratogenic effects at moderate to high doses (0.001-0.1 mg/egg) (Chhibber and Gilani, 1986). However, intravenous injection of acrolein in pregnant rabbits showed no developmental effects in the offspring (Claussen et al., 1980). Based on this latter study, the World Health Organization (1992) concluded that human exposure to acrolein was unlikely to affect the developing embryo.

8. Derivation of Reference Exposure Levels

8.1 Acrolein Acute Reference Exposure Level

Study	Darley et al., 1960
Study population	36 healthy human volunteers
Exposure method	5 min exposure: carbon-filter respirators worn
Exposure continuity	Single exposure
Exposure duration	5 min
Critical effects	subjective ocular irritation
LOAEL	0.06 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	n/a
LOAEL uncertainty factor (UF_L)	6 (default: mild effect, no NOAEL)
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic ($UF_{A\text{-}k}$)	1 (default: human study)
Toxicodynamic ($UF_{A\text{-}d}$)	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic ($UF_{H\text{-}k}$)	1 (site of contact; no systemic effects)
Toxicodynamic ($UF_{H\text{-}d}$)	10 (greater susceptibility of children to asthma exacerbation)
Cumulative uncertainty factor	60
Reference Exposure Level	2.3 μg/m³ (1.0 ppb)

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document).

The study by Darley et al. (1960) was selected as the best available acute exposure study employing human subjects. In addition, the ocular mucosa and the nasal mucosa are both innervated by cranial nerve V (trigeminal nerve). As noted by Doty et al. (2004), numerous studies employing n-alcohols, ketones, alkylbenzenes, terpenes, butyl acetate and toluene, report thresholds for ocular and intranasal irritation to be of the same magnitude suggesting that for most volatiles, tests of ocular and nasal irritancy are of equivalent sensitivity. Thus the endpoint of ocular irritancy used in this study is expected to also reflect irritancy of the upper respiratory tract. Confidence in this REL calculation is moderate as the LOAEL used is based on an estimated LOAEL of 0.06 ppm rather than a measured level. A default uncertainty factor of 6 is associated with the use of a LOAEL for mild effects in the absence of a NOAEL in acute REL derivations (see Section 4.4.5 of the TSD). Due to its high reactivity, the effects of exposure to acrolein in the air are largely confined to the site of contact, in this case the eyes, with negligible or no systemic effects. This localization of effects to the site of contact is supported by the confinement of acrolein's effects to the upper respiratory tract in the animal studies of acute inhalation exposure. Based on modeling of adults and 3-month old children that takes into account age-related ventilation rates and respiratory tract surface area, the deposition kinetics of reactive gases are generally thought not to be greatly different between adults and children

(Ginsberg et al., 2005). Because of this, a value of 1 is used for the kinetic component of the intraspecies uncertainty factor (UF_{H-k}), rather than a more extended value of √10 or 10 which are used where metabolic processes also contribute to inter-individual variability. While ocular irritation is not expected to be substantially different between children and adults, the respiratory irritant effect, with documented potential to exacerbate asthma, is clearly an effect with the potential to differentially impact infants and children. The toxicodynamic component of the intraspecies uncertainty factor UF_{H-d} is therefore assigned an increased value of 10 to account for potential asthma exacerbation. These considerations are applied equally to the acute, 8-hour and chronic REL. Based on this study, an acute REL for acrolein exposure is calculated to be 2.3 $\mu g/m^3$ (1.0 ppb).

As noted in Section 5.1, contact lens wearers may be at greater risk for ocular irritation with acrolein exposure. However, since contact lens users, and infants and children are generally mutually exclusive groups, it is expected that with the ten-fold toxicodynamic UF_{H-d} described above, the acute REL should be adequately protective of these individuals as well.

The acute REL above is supported by a study in humans by Weber-Tschopp et al. (1977). During a 40 minute exposure to increasing concentrations of acrolein, significant ocular irritation was first reported at 0.07 ppm. This represents the LOAEL for this effect and is similar to the LOAEL of 0.06 ppm in Darley et al. (1960). The same uncertainty and adjustment factors, and rationale apply as in Darley, giving an acute REL of 2.7 $\mu g/m^3$ (1.2 ppb).

Study	Weber-Tschopp et al. (1977)
Study population	54 healthy human volunteers
Exposure method	Exposure chamber
Exposure continuity	Increasing concentration (0-0.6 ppm)
Exposure duration	40 min
Critical effects	subjective ocular irritation
LOAEL	0.07 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	n/a
LOAEL uncertainty factor (UF_L)	6 (no NOAEL)
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	60
Reference Exposure Level	2.7 $\mu g/m^3$ (1.2 ppb)

Sensory irritancy is the critical response to acute acrolein exposure. For this effect both the Darley and Weber-Tschopp studies found similar effect levels resulting in similar estimates for the acute REL. In consideration of this, we took the geometric mean of the REL values from these studies to derive the acute REL of **2.5 µg/m^3 (1.1 ppb).**

A similar acute REL was calculated as shown below based on lesions in nasal epithelium in rats exposed to acrolein for 6 hours/day for 3 days (Cassee et al., 1996b). There were sufficient data in this study to permit the application of the BMD method in preference to the NOAEL/LOAEL approach. A $BMCL_{05}$ of 56 µg/m^3 was derived based on the incidence of moderate to severe lesions at each exposure level. Irritancy was not the endpoint in this study so a time adjustment was applied using $C^n * T = K$ (n = 3) to adjust the 18 hours of exposure to 1 hour that gave 147 µg/m^3 (see Section 5.6.1 of the TSD). Interspecies uncertainty factors of 2 for toxicokinetic differences with use of a dosimetric adjustment factor (DAF) of 0.85 (dosimetric adjustment factor – described below and in Section 4.4.7.2.2 of the TSD), and √10 for toxicodynamic variability were combined with a combined intraspecies UF of 10 (1 for kinetic and 10 for dynamic variability, reflecting the expectation of greater toxicodynamic variability) for a cumulative UF of 60 and an acute REL of 0.91 ppb.

Study	Cassee et al., 1996b
Study population	11 rats
Exposure method	Nose-only inhalation
Exposure continuity	6 hr/day
Exposure duration	3 days
Critical effects	lesions of the respiratory epithelium
LOAEL	0.25 ppm (0.58 mg/m^3)
NOAEL	not observed
Benchmark concentration ($BMCL_{05}$)	56 µg/m^3
Time-adjusted exposure	C^n*T n = 3
Extrapolated concentration	147 µg/m^3 $(56^3*6/1*3/1)^{1/3}$
Human concentration adjustment	125 µg/m^3 = 147*0.85 (DAF)
LOAEL uncertainty factor (UF_L)	not applied
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (DAF adjustment with analogue chemical)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	60
Reference Exposure Level	2.1 µg/m^3 (0.91 ppb)

The DAF is a factor derived by OEHHA based on the modeled comparative flux of formaldehyde in the upper respiratory tracts of rats, rhesus monkeys and humans by Kimbell et al. (2001) (see Section 4.4.7.2.2 of the TSD). Kimbell et al. used three-dimensional,

anatomically realistic, computational flow dynamic models to estimate mass flux across 20 consecutive bins representing the nasal passages. The mean flux at each bin was weighted by the percent of non-squamous epithelium in that bin to derive a weighted average flux for each bin. Averaging across all 20 bins provides an overall estimate of the flux for comparison between species (rat, 13.63 pmol/mm^2; human, 30.80 pmol/mm^2). Peak flux values were also estimated for the rat (2620 pmol/mm^2) and human (2082 pmol/mm^2), and averaged with the mean flux values to estimate the DAF (0.85). The DAF is the ratio of this value for the rat to that for humans. Although acrolein is more reactive than formaldehyde, both compounds appear to have their effects primarily on the respiratory (vs. olfactory) epithelium (Cassee et al., 1996a). This supports the assumption that in applying the DAF to acrolein, acrolein and formaldehyde deposit similarly in the nasal passages. In the absence of acrolein-specific modeling data, any residual uncertainty associated with this assumption is reflected in the use of an interspecies $UF_{A\text{-}k}$ of 2.

8.2 Acrolein 8-Hour Reference Exposure Level

Study	Dorman et al., 2008
Study population	360 adult Fischer-344 rats
Exposure method	Discontinuous whole body 0.02 – 1.8 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	65 days
Critical effects	Lesions in the respiratory epithelium
LOAEL	0.6 ppm
NOAEL	0.2 ppm
Benchmark concentration	not derived
Time-adjusted exposure	C * T = K
Extrapolated 8 hour concentration	71 ppb = (0.2*6 /24*5/7*20/10)
Human concentration adjustment	60 ppb = 71*0.85 (DAF)
LOAEL uncertainty factor (UF_L)	1 (NOAEL observed)
Subchronic uncertainty factor (UFs)	√10
Interspecies uncertainty factor	
Toxicokinetic ($UF_{A\text{-}k}$)	2 (DAF adjustment with analogue chemical)
Toxicodynamic ($UF_{A\text{-}d}$)	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic ($UF_{H\text{-}k}$)	1
Toxicodynamic ($UF_{H\text{-}d}$)	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	200
Reference Exposure Level	**0.70 μg/m^3 0.30 ppb)**

The 8-hour Reference Exposure Level is a concentration at or below which adverse non-cancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the TSD).

The 8-hour and chronic RELs are based on the observation of lesions in rat respiratory epithelium by Dorman et al. (2008). In this study, a LOAEL of 0.6 ppm and a NOAEL of 0.2 ppm were observed for respiratory lesions in rats. The observation of a NOAEL eliminates the need for a UF for the LOAEL to NOAEL conversion. The critical effect of lesion formation is not a sensory irritancy effect so a time (T) adjustment was applied using C*T = K to extrapolate from the 6 to 24 hours and from 5 to 7 days. This chronic exposure was converted to an 8 hour

exposure with the 8-hr breathing rate conversion of 20/10 and yields an extrapolated 8 hour concentration of 71 ppb. A human concentration of 60 ppb was estimated using a DAF of 0.85. Although the use of the DAF is expected to correct for pharmacokinetic differences between species, an interspecies kinetic UF of 2 was used because the DAF is based on an analogue (formaldehyde). The default interspecies UF_{A-d} of √10 was applied to compensate for the absence of data on pharmacodynamic differences between species. An intraspecies UF_{H-k} of 1 was used since, although the data are only for adult animals, the pharmacokinetic differences between adult and young animals are not expected to be great based on the similar inhalation dosimetry associated with reactive gases in adults and infants (Ginsberg et al., 2005). The potential pharmacodynamic differences among individuals (especially those with and without asthma) and between adults and infants (due to the immaturity of the infants respiratory tract) are expected to be greater. For example, irritant gases more readily stimulate the hyper-reactive airways of asthmatics while enhanced mucus production in response to irritant gases may more easily block the infant's narrower airways. As described in Section 5.2, exacerbation of asthma by acrolein is expected to disproportionately affect children. For these reasons, an intraspecies UF_{H-d} of 10 was employed. The UF_{H-d} of 10 is the default in the absence of human kinetic data. This resulted in a cumulative UF of 200 and an 8-hour REL of 0.70 μg/m³ (0.30 ppb).

These results are supported by studies of Kutzman et al. (1985) and Feron et al. (1978) following exposure of rats to acrolein for 6 hours/day, 5 days/week for 62 days. In these studies, a LOAEL of 0.4 ppm was observed for nasal lesion formation.

Study	Kutzman et al., 1985; Feron et al. (1978)
Study population	96 adult Fischer-344 rats
Exposure method	Discontinuous whole body 0.4 – 4.0 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	62 days
Critical effects	Lesions in the respiratory epithelium
LOAEL	0.4 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	$C^n * T = K$, where n =1.2
Extrapolated 8 hour concentration	143 ppb = (0.4*6 /24*5/7*20/10)
Human concentration adjustment	122 ppb = 143*0.85 (DAF)
LOAEL uncertainty factor (UF_L)	3 (mild effect; no NOAEL)
Subchronic uncertainty factor (UFs)	√10
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (DAF adjustment with analogue chemical)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	600
Reference Exposure Level	0.46 μg/m³ (0.20 ppb**)**

The experimental designs and results from these two studies were essentially identical. As above, the critical effect of lesion formation is not a sensory irritancy effect so a time (T) adjustment was applied using C*T = K to extrapolate to an 8 hour concentration of 143 ppb. A UF of 3 for the use of a LOAEL reflects the expectation that the NOAEL, while not reported in either of these studies, will not be far from the LOAEL. This is based on the steepness of the dose-response in a plot of the nasal histopathology scoring vs acrolein concentration in the Feron paper, and the observation of a NOAEL three-fold lower than the LOAEL in the Dorman study (0.2 vs 0.6 ppm). An adjusted human concentration of 122 ppb was estimated using a DAF of 0.85. The rest of the uncertainty factors were the same as for the crtitical Dorman study This resulted in a cumulative UF of 600 and an 8-hour REL of 0.46 μg/m³ (0.20 ppb). Thus, this derivation is supportive of the REL derived from Dorman et al. (2008).

8.3 Acrolein Chronic Reference Exposure Level

Study	Dorman et al., 2008
Study population	360 adult Fischer-344 rats
Exposure method	Discontinuous whole body 0.02 – 1.8 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	65 days
Critical effects	Lesions in the respiratory epithelium
LOAEL	0.6 ppm
NOAEL	0.2 ppm
Benchmark concentration	not derived
Time-adjusted exposure	36 ppb = (0.2*6/24*5/7)
Human concentration adjustment	30 ppb = 36*0.85 (DAF)
LOAEL uncertainty factor (UF_L)	1 (NOAEL observed)
Subchronic uncertainty factor (UFs)	√10 (exposure 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (DAF adjustment based on analogue chemical)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	200
Reference Exposure Level	**0.35 μg/m³ (0.15 ppb)**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from continuous chronic exposures (see Section 7 in the Technical Support Document).

The chronic REL was developed using the same study as the 8-hr REL but with a time extrapolation to continuous exposure since the endpoint was not trigeminal irritancy (see Section 1.2.3 in the TSD). It is based on the observed NOAEL of 0.2 ppm for respiratory lesions in rats. The observation of a NOAEL eliminates the need for a UF for the LOAEL to NOAEL conversion. Time adjustment from the experimental to continuous exposure gave 36 ppb (0.2*6 hr/24 hr*5 days/7 days). A DAF of 0.85 gave an equivalent human exposure of 30 ppb. Use of the DAF for an analogue chemical entails an uncertainty factor of 2 as described previously. The

same UFs and rationale as used in the derivation of the 8-hour REL are applied to the chronic REL. The resulting cumulative UF of 200 gave an estimated reference exposure level of 0.35 μg/m³ (0.15 ppb).

These results were supported by those of Kutzman et al. (1985) and Feron et al. (1978)

Study	Kutzman et al., 1985; Feron et al. (1978)
Study population	96 adult Fischer-344 rats
Exposure method	Discontinuous whole body to 0 – 4.0 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	62 days
Critical effects	Lesions in the respiratory epithelium
LOAEL	0.4 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time adjusted exposure	0.071 ppm = 0.4*6/24*5/7
Human concentration adjustment	60 ppb = 0.071 * 0.85 (DAF)
LOAEL uncertainty factor (UF_L)	3 (no NOAEL)
Subchronic uncertainty factor (UFs)	√10 (exposure 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (with DAF adjustment)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	600
Reference Exposure Level	**0.10 μg/m³ (0.04 ppb)**

The LOAEL of 0.4 ppm was adjusted to a continuous exposure of 0.071 ppm (0.4*6 hr/24 hr*5 days/7 days). Application of a DAF of 0.85 gave a human equivalent concentration of 60 ppb (138 $\mu g/m^3$). The UFs applied here are the same as those for the Dorman study with the inclusion of a LOAEL to NOAEL UF of 3. The cumulative UF of 600 gives a chronic REL of 0.10 μg/m³ (0.04 ppb). The study by Dorman et al. was selected in preference to these studies because it identified a NOAEL for the critical effect.

The U.S. EPA (2003) based its RfC of 0.02 $\mu g/m^3$ on the study by Feron et al. (1978) from which a HEC of 0.02 mg/m^3 was derived based on a regional gas dosimetric ratio (RGDR) of 0.14 and an adjusted LOAEL of 0.16 mg/m^3 (0.14 * 0.16 = 0.02). U.S. EPA applied a total uncertainty factor of 1,000 (3 for interspecies extrapolation from a dosimetrically adjusted dose; 10 for intra-human variability; 3 for the use of a LOAEL; 10 for subchronic to chronic extrapolation). In contrast to the RGDR of 0.14, to better account for differences in rat and human exposures to reactive gases, OEHHA used a DAF of 0.85 based on comparative modeling of gas flux in human and rat nasal passages described above. This, combined with UFs of 6 for interspecies uncertainty (2 for use of the DAF, √10 for toxicodynamic differences), √10 for the use of a subchronic study, and 3 for the use of a LOAEL (vs US EPA's 3, 3, and 10, respectively) account for the difference between the REL and the U.S. EPA RfC.

For comparison, the state of Minnesota Department of Health reports a subchronic Health Risk Value (HRV) for acrolein of 0.2 $\mu g/m^3$, a level thought to be without significant risk following inhalation exposure for 13 weeks (MDH, 2002).

8.4 Acrolein as a Toxic Air Contaminant

Acrolein was identified by the ARB as a toxic air contaminant (TAC) in accordance with section 39657(b) of the California Health and Safety Code on April 8, 1993 (Title 17, California Code of Regulations, Section 93001)(CCR, 2007). In view of the differential impacts on infants and children identified in Section 6.2 (more severe effects associated with bronchoconstriction and asthma exacerbation, less ability to escape or avoid exposure), OEHHA listed acrolein as a TAC which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c) .

9. References

Astry CL and Jakab GJ (1983). The effects of acrolein exposure on pulmonary antibacterial defenses. Toxicol Appl Pharmacol 67(1): 49-54.

Borchers MT, Carty MP and Leikauf GD (1999a). Regulation of human airway mucins by acrolein and inflammatory mediators. Am J Physiol 276(4 Pt 1): L549-55.

Borchers MT, Wert SE and Leikauf GD (1998). Acrolein-induced MUC5ac expression in rat airways. Am J Physiol 274(4 Pt 1): L573-81.

Borchers MT, Wesselkamper S, Wert SE, Shapiro SD and Leikauf GD (1999b). Monocyte inflammation augments acrolein-induced Muc5ac expression in mouse lung. Am J Physiol 277(3 Pt 1): L489-97.

Bouley G, Dubreuil A, Godin J, Boisset M and Boudene C (1976). Phenomena of adaptation in rats continuously exposed to low concentrations of acrolein. Ann Occup Hyg 19(1): 27-32.

Bouley G, Dubreuil A, Godin J and Boudene C (1975). [Effects in the rat of a weak dose of acrolein inhaled continuously]. Eur J Toxicol Environ Hyg 8(5): 291-7.

Buckley LA, Jiang XZ, James RA, Morgan KT and Barrow CS (1984). Respiratory tract lesions induced by sensory irritants at the RD50 concentration. Toxicol Appl Pharmacol 74(3): 417-29.

CARB (2005b). Annual Statewide Toxics Summary - acrolein Sacramento, CA. http://www.arb.ca.gov/adam/toxics/statepages/acrostate.html.

Cassee FR, Arts JH, Groten JP and Feron VJ (1996a). Sensory irritation to mixtures of formaldehyde, acrolein, and acetaldehyde in rats. Arch Toxicol 70(6): 329-37.

Cassee FR, Groten JP and Feron VJ (1996b). Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde, and acrolein. Fundam Appl Toxicol 29(2): 208-18.

CCR (2007). California Code of Regulations Section 93001 Hazardous Air Pollutants Identified as Toxic Air Contaminants. Sacramento, CA: California Office of Administrative Law. 8-20-07. http://ccr.oal.ca.gov/linkedslice/default.asp?SP=CCR-1000&Action=Welcome.

Chhibber G and Gilani SH (1986). Acrolein and embryogenesis: an experimental study. Environ Res 39(1): 44-9.

Claussen U, Hellmann W and Pache G (1980). The embryotoxicity of the cyclophosphamide metabolite acrolein in rabbits, tested in vivo by i.v. injection and by the yolk-sac method. Arzneimittelforschung 30(12): 2080-3.

Darley E, Middleton J and Garber M (1960). Plant damage and eye irritation from ozone-hydrocarbon reactions. Agricul Food Chem 8(6): 483-484.

Dorman DC, Struve MF, Wong BA, Marshall MW, Gross EA and Willson GA (2008). Respiratory tract responses in male rats following subchronic acrolein inhalation. Inhal Toxicol 20(3): 205-16.

Doty RL, Cometto-Muniz JE, Jalowayski AA, Dalton P, Kendal-Reed M and Hodgson M (2004). Assessment of upper respiratory tract and ocular irritative effects of volatile chemicals in humans. Crit Rev Toxicol 34(2): 85-142.

Feron VJ and Kruysse A (1977). Effects of exposure to acrolein vapor in hamsters simultaneously treated with benzo[a]pyrene or diethylnitrosamine. J Toxicol Environ Health 3(3): 379-94.

Feron VJ, Kruysse A, Til HP and Immel HR (1978). Repeated exposure to acrolein vapour: subacute studies in hamsters, rats and rabbits. Toxicology 9(1-2): 47-57.

Finkelstein EI, Nardini M and van der Vliet A (2001). Inhibition of neutrophil apoptosis by acrolein: a mechanism of tobacco-related lung disease? Am J Physiol Lung Cell Mol Physiol 281(3): L732-9.

Ginsberg GL, Foos BP and Firestone MP (2005). Review and analysis of inhalation dosimetry methods for application to children's risk assessment. J Toxicol Environ Health A 68(8): 573-615.

Gosselin B, Wattel F, Chopin C, Degand P, Fruchart JC, Van der Loo D and Crasquin O (1979). [A case of acute acrolein poisoning]. Nouv Presse Med 8(30): 2469-72.

Grafstrom RC, Sundqvist K, Dypbukt JM and Harris CC (1987). Pathobiological effects of aldehydes in cultured human bronchial cells. IARC Sci Publ(84): 443-5.

Gurtoo HL, Marinello AJ, Struck RF, Paul B and Dahms RP (1981). Studies on the mechanism of denaturation of cytochrome P-450 by cyclophosphamide and its metabolites. J Biol Chem 256(22): 11691-701.

IARC (1995). Dry Cleaning, Some Chlorinated Solvents and Other Industrial Chemicals. IARC monographs 63: 337-371.

Kane LE, Barrow CS and Alarie Y (1979). A short-term test to predict acceptable levels of exposure to airborne sensory irritants. Am Ind Hyg Assoc J 40(3): 207-29.

Kimbell JS, Godo MN, Gross EA, Joyner DR, Richardson RB and Morgan KT (1997). Computer simulation of inspiratory airflow in all regions of the F344 rat nasal passages. Toxicol Appl Pharmacol 145(2): 388-98.

Kimbell JS, Overton JH, Subramaniam RP, Schlosser PM, Morgan KT, Conolly RB and Miller FJ (2001). Dosimetry modeling of inhaled formaldehyde: Binning nasal flux predictions for quantitative risk assessment. Toxicological Sciences 64(1): 111-121.

Kutzman R. (1981). A subchronic inhalation study of Fischer 344 rats exposed to 0, 0.4, 1.4, or 4.0 ppm acrolein. National Toxicology Program: Interagency Agreeement No. 222-Y01-ES-9-0043. Brookhaven National Laboratory. Upton, NY

Kutzman RS, Popenoe EA, Schmaeler M and Drew RT (1985). Changes in rat lung structure and composition as a result of subchronic exposure to acrolein. Toxicology 34(2): 139-51.

Lam CW, Casanova M and Heck HD (1985). Depletion of nasal mucosal glutathione by acrolein and enhancement of formaldehyde-induced DNA-protein cross-linking by simultaneous exposure to acrolein. Arch Toxicol 58(2): 67-71.

Leikauf GD, Doupnik CA, Leming LM and Wey HE (1989a). Sulfidopeptide leukotrienes mediate acrolein-induced bronchial hyperresponsiveness. J Appl Physiol 66(4): 1838-45.

Leikauf GD, Leming LM, O'Donnell JR and Doupnik CA (1989b). Bronchial responsiveness and inflammation in guinea pigs exposed to acrolein. J Appl Physiol 66(1): 171-8.

Linhart I, Frantik E, Vodickova L, Vosmanska M, Smejkal J and Mitera J (1996). Biotransformation of acrolein in rat: excretion of mercapturic acids after inhalation and intraperitoneal injection. Toxicol Appl Pharmacol 136(1): 155-60.

Lyon JP, Jenkins LJ, Jr., Jones RA, Coon RA and Siegel J (1970). Repeated and continuous exposure of laboratory animals to acrolein. Toxicol Appl Pharmacol 17(3): 726-32.

Mahut B, Delacourt C, de Blic J, Mani TM and Scheinmann P (1993). Bronchiectasis in a child after acrolein inhalation. Chest 104(4): 1286-7.

Mannino DM, Homa DM, Pertowski CA, Ashizawa A, Nixon LL, Johnson CA, Ball LB, Jack E and Kang DS (1998). Surveillance for asthma--United States, 1960-1995. MMWR CDC Surveill Summ 47(1): 1-27.

McNulty M, Heck HD and Csasnova-Schmitz M (1984). Depletion of glutathione in rat respiratory mucosa by inhaled acrolein. Fed Proc 43: 575, abstract 1695.

MDH (2002). Health Risk Values for Air - Subchronic HRVs March 2002. Minnesota Department of Health,. http://www.health.state.mn.us/divs/eh/air/hrvtable.htm#sub.

Morris JB, Symanowicz PT, Olsen JE, Thrall RS, Cloutier MM and Hubbard AK (2003). Immediate sensory nerve-mediated respiratory responses to irritants in healthy and allergic airway-diseased mice. J Appl Physiol 94(4): 1563-71.

Nazaroff WW and Singer BC (2004). Inhalation of hazardous air pollutants from environmental tobacco smoke in US residences. J Expo Anal Environ Epidemiol 14 Suppl 1: S71-7.

NTIS. (1981). Acrolein health effects. Final task 6 report. Contract # 68-03-2928. National Technical Information Service. Arlington (VA)

OEHHA. (2001). *Prioritization of toxic air contaminants - children's health protection act - final report*. Available on-line at: <http://www.oehha.ca.gov/air/toxic_contaminants/SB25finalreport.html>.

Parent RA, Paust DE, Schrimpf MK, Talaat RE, Doane RA, Caravello HE, Lee SJ and Sharp DE (1998). Metabolism and distribution of [2,3-14C]acrolein in Sprague-Dawley rats. II. Identification of urinary and fecal metabolites. Toxicol Sci 43(2): 110-20.

Penn A, Nath R, Pan J, Chen L, Widmer K, Henk W and Chung FL (2001). 1,N(2)-propanodeoxyguanosine adduct formation in aortic DNA following inhalation of acrolein. Environ Health Perspect 109(3): 219-24.

Prentiss A (1937). Chemicals in War. In. McGraw-Hill. New York: 139-140.

Roemer E, Anton HJ and Kindt R (1993). Cell proliferation in the respiratory tract of the rat after acute inhalation of formaldehyde or acrolein. J Appl Toxicol 13(2): 103-7.

Roux E, Hyvelin JM, Savineau JP and Marthan R (1999). Human isolated airway contraction: interaction between air pollutants and passive sensitization. Am J Respir Crit Care Med 160(2): 439-45.

Schroeter JD, Kimbell JS, Gross EA, Willson GA, Dorman DC, Tan YM and Clewell HJ, 3rd (2008). Application of physiological computational fluid dynamics models to predict interspecies nasal dosimetry of inhaled acrolein. Inhal Toxicol 20(3): 227-43.

Slott VL and Hales BF (1986). The embryolethality and teratogenicity of acrolein in cultured rat embryos. Teratology 34(2): 155-63.

Struve MF, Wong VA, Marshall MW, Kimbell JS, Schroeter JD and Dorman DC (2008). Nasal uptake of inhaled acrolein in rats. Inhal Toxicol 20(3): 217-25.

Takabe W, Niki E, Uchida K, Yamada S, Satoh K and Noguchi N (2001). Oxidative stress promotes the development of transformation: Involvement of a potent mutagenic lipid peroxidation product, acrolein. Carcinogenesis (Oxford) 22(6): 935-941.

Tanaka K, Nishiyama K, Yaginuma H, Sasaki A, Maeda T, Kaneko SY, Onami T and Tanaka M (2003). [Formaldehyde exposure levels and exposure control measures during an anatomy dissecting course]. Kaibogaku Zasshi 78(2): 43-51.

U.S.EPA. (2003). Toxicological review of acrolein. EPA/635/R-03/003. U.S. Environmental Protection Agency. Washington DC

Weber-Tschopp A, Fischer T, Gierer R and Grandjean E (1977). [Experimentally induced irritating effects of acrolein on men (author's transl)]. Int Arch Occup Environ Health 40(2): 117-30.

WHO. (1992). *Environmental health criteria 127. Acrolein*. World Health Organization. www.inchem.org/documents/ehc/ehc/ehc127.htm#SectionNumber:10.1.

Yang XM, Wu XL, Choi YE, Kern JC and Kehrer JP (2004). Effect of acrolein and glutathione depleting agents on thioredoxin. Toxicology 204(2-3): 209-218.

Inorganic Arsenic Reference Exposure Levels

1. Summary

Acute, 8-hour and chronic reference exposure levels (RELs) were derived for inorganic arsenic including arsine. Inorganic arsenic causes a wide variety of toxic effects in humans and experimental animals including effects on development, the vascular system, the nervous system, blood, lung, and skin. The most sensitive acute effects were seen in mice (fetal development) whereas the most sensitive 8-hour and chronic effects were decreased intellectual function in children. The relevant literature evaluated in this assessment was published before April 1, 2008. The key values are summarized below.

1.1 Inorganic Arsenic Acute REL

Reference Exposure Level	**0.2 μg As/m³**
Critical effect(s)	Decreased fetal weight in mice
Hazard Index target(s)	Development (teratogenicity); cardiovascular system; nervous system

1.2 Inorganic Arsenic 8-Hour REL

Reference Exposure Level	**0.015 μg/ As/m³**
Critical effect(s)	Decreased intellectual function in 10 year old children
Hazard Index target(s)	Development; cardiovascular system; nervous system; lung; skin

1.3 Inorganic Arsenic Chronic REL

Reference Exposure Level	**0.015 μg As/m³**
Oral Reference Exposure Level	0.0035 μg/kg bw-day
Critical effect(s)	Decreased intellectual function in 10 year old children
Hazard Index target(s)	Development; cardiovascular system; nervous system; lung; skin

2. Physical & Chemical Properties

Table 2.1 Arsenic and Arsenic Species*

Molecular formula	Molecular weight	Percent As by weight	Synonyms	CAS Registry Number
As	74.92	100%	Arsenic black, metallic arsenic	7440-38-2
As_2O_3 As_4O_6	197.82 395.68	75.7%	Arsenious oxide, arsenic (III) trioxide, arsenic oxide, arsenous acid, arsenous acid anhydride, Crude Arsenic, White Arsenic	1327-53-3
$AsCl_3$	181.28	41.3%	Arsenic butter, trichloroarsine, arsenious chloride	7784-34-1
As_2O_5	229.82	65.2%	Arsenic pentoxide, arsenic anhydride, arsenic oxide, arsenic acid anhydride	1303-28-2
$AsHNa_2O_4$	185.91	40.3%	Arsenic acid disodium salt, disodium arsenate, sodium arsenate dibasic	7778-43-0
$AsHNa_2O_3$	130.92	57.2%	Arsenous acid disodium salt, arsenious acid sodium salt	7784-46-5
AsH_3	77.94	96.12	Arsine, arsane, arsenic hydride, arsenous hydride, hydrogen arsenide, arsenic trihydride	7784-42-1
$As(OH)_3$	125.94	59.49	Arsenous acid	13464-58-9
$AsO(OH)_3$	141.93	52.78	Arsenic acid, orthoarsenic acid	7778-39-4
As_4S_4	427.92	70.03	Arsenic disulfide, realgar, red arsenic sulfide	
$CH_3AsO(OH)_2$	139.97	53.51	Monomethylarsonic acid	124-58-3
$CH_3As(OH)_2$	123.77	60.41	Monomethylarsonous acid	25400-23-1
$(CH_3)_2AsO(OH)$	137.99	54.28	Dimethylarsinic acid, cacodylic acid	75-60-5
$(CH_3)_2AsOH$	121.99	61.40	Dimethylarsinous acid	55094-22-9
$(CH_3)_3AsO$	136.02	55.06	Trimethylarsine oxide	4964-14-1

*Note: Methylated arsenic species occurring naturally and as metabolites (IARC, 2004)

2.1 Arsenic (Metallic) (ATSDR, 2000)

Description	Yellow, black or gray solid
Molecular formula	see Table 2.1
Molecular weight	see Table 2.1
Specific gravity (water = 1)	5.778 g/cm^3 @ 25°C
Boiling point	613°C (sublimes) at 760 mm Hg
Vapor pressure	7.5 x 10^{-3} mmHg at 280 °C
Flashpoint	not applicable
Explosive limits	not applicable
Solubility	soluble in nitric acid, insoluble in water
Odor threshold	not applicable
Odor description	odorless
Metabolites	dimethylarsinic acid, methylarsonic acid
Conversion factor	not applicable for As

2.2 Arsenic Trioxide (ATSDR, 2000)

Description	As_2O_3: White solid, glassy, amorphous lumps or crystal
Molecular formula	See Table 2.1
Molecular weight	197.84
Density	As_2O_3: 3.865 g/cm^3
Boiling point	As_2O_3: 460°C
Melting point	As_2O_3: 274°C
Solubility	Oxides: slightly soluble in water 17g/L, insoluble in alcohol, chloroform, ether.
Metabolites	Dimethylarsinic acid, methylarsonic acid

2.3 Arsine (U.S. EPA, 2006a)

Description	Colorless gas
Molecular formula	AsH_3
Molecular weight	77.93
Specific gravity (Water = 1)	1.689 @ 84.9°C
Boiling point	-62.55°C
Melting point	-117°C
Vapor pressure	Greater than 1 atm
Vapor density (Air = 1)	2.695
Solubility	soluble in chloroform and benzene, slightly soluble in water (20 mL/100 mL at 20 C), ethyl alcohol and in alkalis
Odor threshold	0.5 ppm
Odor description	garlic-like or fishy odor
Metabolites	oxidation to arsenite, arsenate, other unidentified (Landrigan *et al*., 1982; Carter *et al*., 2003)
Conversion factor	1 ppm = 3.19 mg/m³ @ 25°C

3. Occurrence and Major Uses

Arsenic is ubiquitous and is found in small amounts in soils and water throughout the world and also in foods, particularly seafood (NIOSH, 1975). Ore refining processes, including the smelting of copper and lead, are the major sources of release of arsenic dust and inorganic arsenic compounds. Arsenic trioxide is the form of inorganic arsenic most commonly produced. It is used as a raw material for the production of other inorganic arsenic compounds (Asi), alloys, and organic arsenic compounds (Grayson, 1978).

Pesticides have historically constituted the largest single use (50%) of arsenic compounds (HSDB, 1995). The major arsenic herbicides manufactured are monosodium methyl arsonate (MSMA), disodium methyl arsonate (DSMA), and dimethyl arsenic acid (cacodylic acid). Inorganic arsenic compounds are also used as herbicides (arsenite), insecticides (arsenic trioxide, calcium and other arsenates), or rodenticides (sulfides) (ACGIH, 1992). Arsenic trichloride, for example, is used mainly as a chemical intermediate in the production of insecticides, but has other applications in the ceramics and pharmaceutical industries (HSDB, 1995). Arsenic was used as a pesticide to treat tobacco; thus, cigarette smoke was another common source of exposure (U.S.EPA, 1984). The use of arsenic compounds in agriculture has reduced in recent years and U.S. EPA is considering ending their uses under the pesticide reregistration program (U.S. EPA, 2006b).

Arsenic-based wood preservatives have constituted the next largest use (40%) of arsenic compounds (HSDB, 1995). In December 2003 the U.S. EPA terminated all residential uses of wood preservatives containing arsenic limiting such products to restricted use by certified pesticide applicators (U.S. EPA, 2002).

The highly toxic trivalent arsenic compounds, such as arsenic trioxide, are typically introduced into the environment as a result of industrial processes including the smelting of metal ores. Pentavalent arsenic compounds are generally considered to be less toxic and are most frequently found naturally.

Processes such as smelting, galvanizing, soldering, and etching, that require the treatment of metal with strong acids, are possible sources of arsine gas. Acid treatment of metals contaminated with arsenic can result in the release of arsine gas. Arsine is used to provide arsenic as an ingredient in semiconductor manufacture. Combustion of fossil fuels may produce arsine gas.

4. Toxicokinetics

A knowledge of the metabolism of inorganic arsenic has long been thought to be essential to understanding the mode(s) of action of inorganic arsenic toxicity. Trivalent (+3, As^{III}) arsenic species (e.g., arsenite) have often exhibited greater acute toxicity than pentavalent (+5, As^{V}) species (e.g., arsenate). The terms arsenite and arsenate refer to the ionized anions of arsenous acid and arsenic acid, respectively, as they exist in aqueous solution at physiological pH. Since the metabolism of inorganic arsenic in mammalian species generally proceeds via alternate reductive and oxidative methylation steps to mono- (MMA) and dimethyl (DMA) arsenic acids, it was believed that methylation represented detoxication of inorganic arsenic. However, recent

evidence supports the idea that trivalent methylated species are in some cases more toxic than inorganic precursors and may play a key role in arsenic toxicity for selected endpoints. The metabolism of arsine (As^{-III}), while less studied, appears to progress similarly after its oxidation to arsenite (As^{V}) and is in part the basis for including arsine in the RELs for inorganic arsenic.

Several comprehensive reviews of the absorption, distribution, metabolism and elimination of arsenic have been published (Vahter, 1983; Thompson, 1993; ATSDR, 2000; NRC, 2001). Most information on the toxicokinetics of arsenic derives from oral exposure studies. The kinetics of arsenic varies depending on the chemical form of arsenic and on the animal species. The following discussion is limited to the oxidized forms found in water and air and forms that are ingested via the aquatic food chain. These include the inorganic, soluble forms of arsenite (As^{III}) and arsenate (As^{V}), as well as the organic monomethylarsonate (MMA), dimethylarsinic acid (DMA), trimethylarsine (TMA), and or arsenobetaine (in fish).

4.1 Inorganic Arsenic Oxides

Owen (1990) reported inhalation absorption of 32 percent (range 30 to 34 %) from arsenic containing aerosols, however it is uncertain if this figure included the gastrointestinal absorption of arsenic particles from the upper respiratory tract. The International Commission on Radiological Protection Human Respiratory Tract Model (ICRP, 1994) gives total deposition fractions for 10 yr old children inhaling 1 μm activity median thermodynamic diameter particles at 0.31 to 2.03 m^3/hr of 0.42 to 0.58. There are relatively few data on the kinetics of airborne arsenic excretion. Mann *et al*. (1996a) modeled inhalation exposures based on the occupational data of Vahter *et al*. (1986) and Offergelt *et al*. (1992). For simulated occupational exposures of 10 μg/m^3 of arsenic aerosol of MMAD of 5.0 μm, GSD of 2.1, 1.2 L tidal volume and a breathing rate of 16 /min, urinary excretion increased over the work week's exposure from 7 to 25 μg As/g creatinine.

The MMAD refers to the mass median aerodynamic diameter and GSD the geometric standard deviation. These values characterize a distribution of particles in an aerosol. The units refer to the first order rate constant for the absorption of arsenic into the blood plasma from the model lung compartments. The model has separate compartments for the nasopharynx, tracheobroncheal, and pulmonary regions of the lung. Deposition of particles in these lung compartments, in units of μg/hr, depends on breathing rate, tidal volume, concentration of particles in the air, and their aerodynamic diameters. Absorption of deposited particles into blood plasma is first order but depends upon the surface area of the region in question, hence the units of /cm^2-hr.

Model predictions of arsenic metabolites (Asi, MMA, DMA) in postshift urine generally fell within the range of observations for 18 workers in the exposure range of 10-1000 μg As/m^3. After daily inhalation exposure of 100 μg As (III)/m^3 for three weeks, the model predictions for urinary metabolite distribution closely matched observed values (predicted/observed means: Asi, 1.05; MMA, 1.0; DMA, 1.0). From the model, Mann *et al*. (1996b) derived a fitted lung absorption first order rate constant for arsenic trioxide dust of 0.01/cm^2-hr.

In general, investigations that have monitored arsenic excretion of experimental animals following parenteral administration have demonstrated that only a small fraction of the

administered arsenic is excreted in the feces. Thus, to estimate the amount of inorganic arsenic absorbed following oral administration, most kinetic and metabolic studies have monitored the urine. Soluble compounds of inorganic arsenic, whether in the trivalent or pentavalent form, are readily absorbed (80-90 percent) in most animal species following oral administration (Charbonneau *et al*., 1978; Vahter, 1981; Hughes *et al*., 1994; Freeman *et al*., 1995). However, only about 40-50 percent absorption has been reported in hamsters (Yamauchi and Yamamura, 1985; Marafante and Vahter, 1987). Absorption of orally administered inorganic arsenic in humans has been shown to range between 54-80 percent (Tam *et al*., 1979; Buchet *et al*., 1981b; a; Kurttio *et al*., 1998).

Inorganic arsenic compounds are poorly absorbed through the skin (Ca.1-5%); the trivalent is more rapidly absorbed than the pentavalent (Wester *et al*., 1993; Wester *et al*., 2004).

Organic forms of arsenic are also extensively absorbed from the gastrointestinal tract. Experimental studies examining the absorption of MMA, DMA, TMA and arsenobetaine in humans have demonstrated 75-92 percent absorption. At low-level exposures, excretion of arsenic and its metabolites seems to balance absorption of inorganic arsenic. With increasing arsenic intake, there is suggestive evidence that methylation appears less complete. Studies, which examine the effect of dose on excretion patterns, have been conducted in mice and humans (Buchet *et al*., 1981b; a; Vahter, 1981). As the dose of inorganic arsenic increases, the percent of arsenic excreted as DMA decreases, accompanied by an increased excretion in the percent as inorganic arsenic. The percent excreted as MMA remains virtually unchanged. *In vitro* metabolism studies on the methylation of inorganic arsenic have demonstrated that the liver is the site of methylating activity and that S-adenosylmethionine and reduced glutathione are required as methyl donors (Buchet and Lauwerys, 1985; 1987).

While absorption from the gastrointestinal tract is the most important route of exposure for waterborne arsenic, some potential for dermal absorption has been reported. Rahman *et al*. (1994) conducted *in vitro* studies with sodium [^{74}As] arsenate and clipped full-thickness mouse skin in a flow-through system. Doses of 5, 50, 500, or 5000 ng were applied to 0.64 cm^2 of skin as a solid, in aqueous vehicle, or in soil. Absorption of sodium arsenate increased linearly with applied dose from all vehicles. The maximum absorption of 62 percent of applied dose was obtained with the aqueous vehicle and the least (0.3 percent) with soil. Wester *et al*. (1993) evaluated the percutaneous absorption of [^{73}As] arsenate from soil or water *in vivo* in Rhesus monkeys and *in vitro* in human cadaver skin. Water solutions of [^{73}As] arsenate at low (0.024 ng/cm^2) or high (2.1 $\mu g/cm^2$) surface concentrations were compared. With topical administration for 24 hr, *in vivo* absorption in the Rhesus monkey was 6.4 ± 3.9 (SD) percent from the low dose and 2.0 ± 1.2 (SD) percent from the high dose. *In vitro* percutaneous absorption of the low dose from water in human skin was 0.93 ± 1.1 percent in receptor fluid and 0.98 ± 0.96 percent in the washed skin; the total was about 1.9 percent. Absorption from soil (0.4 ng/cm^2) was less, at 6.4 percent in the monkey *in vivo* and 0.8 percent in human skin *in vitro*.

The retention and distribution patterns of arsenic are in part determined by its chemical properties. Arsenite (As^{III}) reacts and binds to sulfhydryl groups while arsenate (As^{V}) has chemical properties similar to those of phosphate. As^{V} also has affinity for sulfhydryl groups; however, its affinity is approximately 10-fold less than As^{III} (Jacobson-Kram and Montalbano,

1985). The distribution and retention patterns of As^{III} and As^{V} are also affected by species, dose level, methylation capacity, valence form, and route of administration.

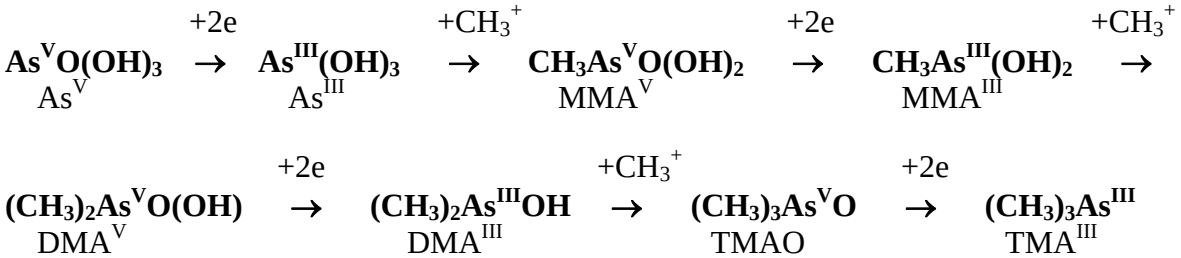
Vahter *et al*. (1984) studied tissue distribution and retention of ^{74}As-DMA in mice and rats. About 80 percent of an oral dose of 0.4 mg As/kg was absorbed from the gastrointestinal tract. In mice >99 percent of the dose was excreted within 3 d compared to only 50 percent in rats, due largely to accumulation in blood, which delayed excretion. Tissue distribution in mice showed the highest initial (0.5-6 hr) concentrations in kidneys, lungs, intestinal mucosa, stomach, and testes. Tissues with the longest retention times were lungs, thyroid, intestinal walls, and lens.

The effect of dose on arsenate disposition was evaluated in adult female $B6C3F_1$ mice dosed orally with 0.5 to 5000 μg/kg [^{73}As]-arsenate in water (Hughes *et al*., 1994). Urine was collected at several time points over a 48-hr period, and feces at 24 and 48 hr post-exposure. The recovery of As-derived radioactivity in excreta and tissues ranged from 83.1 to 89.3 percent of dose. As-derived radioactivity was detected in several tissues (urinary bladder, gall bladder, kidney, liver, lung) although the sum for each exposure level was very low (<0.5 percent of dose). The principal depot was the liver, followed by the kidneys. As the dose of arsenate increased there was a significant increase in the accumulation of radioactivity in the urinary bladder, kidney, liver, and lungs. The greatest concentration of As radioactivity was in the urinary bladder.

Most studies of arsenic metabolism have involved administration of inorganic arsenic (Asi) as arsenate (As^{V}) or arsenite (As^{III}) to an experimental animal or a human, and detection of Asi and the methylated metabolites methylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}) in urine, feces, and tissues.

Thompson (1993) conducted an extensive review and analysis of the mammalian metabolic data on arsenic. The metabolism of arsenate can be viewed as a cascade of reductive and oxidative methylation steps leading successively to As^{III}, MMA^{V}, MMA^{III}, DMA^{V}, DMA^{III}, $TMAO^{V}$, and TMA as outlined in Scheme 1. Recently Hayakawa *et al*. (2005) proposed a new metabolic pathway for arsenite, which does not involve oxidative methylation but rather is mediated by As-glutathione complexes, S-adenosylmethionine (SAM) and human arsenic methyltransferase Cyt19. In this pathway arsenic triglutathione ($As(SG)_3$) is converted to monomethyl-(MADG) and dimethyl-(DMAG) conjugates which are hydrolyzed to MMA^{III} and DMA^{III}, respectively. Thus pentavalent methylated metabolites might arise via oxidation of their trivalent forms rather than the reverse as shown in Scheme 1.

Scheme 1. Biomethylation of Arsenic Involving Alternate Reduction of Pentavalent Arsenic to Trivalent Arsenic Followed by Oxidative Addition of a Methyl Group (after Jiang et al. (2003))



MMA^{III} and DMA^{III} have only recently been detected as stable urinary metabolites in human subjects (Aposhian *et al*., 2000a; Aposhian *et al*., 2000b; Le *et al*., 2000a; Le *et al*., 2000b), and trimethylarsine oxide (TMAO) and trimethylarsine (TMA) are rarely seen and are very minor metabolites in most mammals if found at all. Few data are available on the tissue concentrations of trivalent methylated As species (Kitchin, 2001). Gregus *et al*. (2000) found that in bile duct-cannulated rats, As^{III} and its metabolites were preferentially excreted into bile (22 percent) versus eight percent into urine in two hr. Arsenite appeared in bile rapidly and constituted the large majority in the first 20 min. Thereafter As^{III} declined and MMA^{III} output gradually increased. From 40 min after i.v. As^{III} administration, MMA^{III} was the dominant form of biliary arsenic. Within two hr 9.2 percent of the dose was excreted in the bile as MMA^{III}. Injection of arsenate produced a mixture of As^{V}, As^{III} and MMA^{III} in the bile. Curiously, rats injected with MMA^{V} did not excrete MMA^{III}.

The metabolism results of Styblo *et al*. (1995) in rat liver cytosol *in vitro* seem to support the overall metabolic scheme noted above; MMA^{III} and MMA^{III}-diglutathione complex are more rapidly methylated to the dimethyl forms than MMA^{V}. Thompson also suggests that the data support the presence of two inhibitory loops: (1) competitive inhibition by MMA^{III} of the $As^{III} \rightarrow MMA^{V}$ step catalyzed by monomethyltransferase (MMTase); and (2) possibly noncompetitive inhibition by As^{III} of the $MMA^{III} \rightarrow DMA^{V}$ step catalyzed by dimethyltransferase (DMTase).

Styblo *et al*. (1996) observed 50 μ*M* arsenite inhibition of DMA^{V} production in rat liver cytosol *in vitro*. Healy *et al*. (1998) studied the activity of MMTase in tissues of mice. The activity was determined with sodium arsenite and *S*-[methyl-^{3}H]-adenosyl-L-methionine by measuring the formation of [methyl-^{3}H] monomethylarsonate. The mean MMTase activities (units/mg ± SEM) measured in cytosol of mouse tissues were: liver, 0.40 ± 0.06; testis, 1.45 ± 0.08; kidney, 0.70 ± 0.06; and lung, 0.22 ± 0.01. When mice were given arsenate in drinking water for 32 or 92 days at 25 or 2500 μg As/L, the MMTase activities were not significantly increased compared to controls. MMTases and DMTases have been partially purified from the livers of rabbits (Zakharyan *et al*., 1995), Rhesus monkeys (Zakharyan *et al*., 1996) and hamsters (Wildfang *et al*., 1998). All of the enzyme preparations exhibited Michaelis-Menten enzyme kinetics with Km values ranging from $8x10^{-4}$ *M* for hamster DMTase to $1.8x10^{-6}$ *M* for hamster MMTase. Vmax values ranged from 0.007 pmol/mg protein/hr for hamster DMTase to 39.6 pmol/mg protein/hr for rabbit MMTase. Comparative studies have shown several species to be deficient in methyltransferase activities, notably New World monkeys, marmosets, tamarin, squirrel, chimpanzee, and guinea pig (Vahter *et al*., 1995b; Aposhian, 1997). While comparisons with human arsenic methyl transferase are limited by lack of a purified human enzyme, based on excretion profiles of urinary metabolites the rabbit and hamster appear most pharmacokinetically similar to humans than the other species studied. Walton *et al*. (2003) compared the methylation of arsenite by rat and human primary hepatocytes in vitro (control values in their Tables 1 and 2). For the rat the methylation rate after a 3 hr incubation with 0.1 μM arsenite was 99.3 ± 1.87 pmol CH_3/hr/10^6 cells (mean ± SD, N =4). The human hepatocytes similarly exposed for 24 hr had a methylation rate of 1.68 ± 0.24 pmol CH_3/hr/10^6 cells, over a 50-fold difference in apparent methylation rate.

While the reduction of arsenate and MMA^{V} can be accomplished nonenzymatically *in vitro*, and arsenate reduction by glutathione occurs in mammalian blood *in vivo* (Vahter and Envall, 1983;

Winski and Carter, 1995), these reductive steps are most likely enzymatically mediated *in vivo*. An arsenate reductase has been partially purified from human liver and described (Radabaugh and Aposhian, 2000). The approximate mass of the enzyme was 72,000. It was specific for arsenite (i.e., did not reduce [^{14}C] MMA^V) and exhibited substrate saturation at about 300 μM. The human arsenate reductase requires a thiol and a heat-stable cofactor and is apparently distinct from those isolated from bacteria (Ji and Silver, 1992; Gladysheva *et al*., 1994; Krafft and Macy, 1998).

Monomethyl arsonate (MMA^V) reductases have been isolated and described for rabbit (Zakharyan and Aposhian, 1999) and hamster (Sampayo-Reyes *et al*., 2000). In the latter study the distribution of MMA^V reductase activity was 91.4 nmol MMA^{III}/mg protein/hr in brain and 61.8 nmol MMA^{III}/mg protein/hr in bladder. Skin, kidney and testis all had less than 15 nmol/mg/hr. Spleen, liver, lung, and heart were all between 15 and 62 nmol/mg/hr. The high activity of MMA^V reductase in brain is curious and may help explain some of the neurotoxic effects of arsenic. Due to relatively low affinity of the MMA^V reductase ($K_M = 2.2x10^{-3}$ M) compared to the methyl transferases ($K_M = 5\text{-}9x10^{-6}$ M), the MMA^V reduction is thought to be the rate-limiting step in arsenic metabolism (Zakharyan and Aposhian, 1999). The partially purified human liver MMA^V reductase has been shown to be identical with human glutathione S-transferase Omega class hGSTO 1-1 (Zakharyan *et al*., 2001).

DMA is the main metabolite found in the tissues and urine of most experimental animals administered inorganic arsenic. Humans are also somewhat unique in that MMA has been found to be an important metabolite of inorganic arsenic in addition to DMA. Studies conducted on human volunteers given a single oral dose of inorganic arsenic demonstrated that within 4-7 days, 46-62 percent of the dose was excreted in the urine (Tam *et al*., 1979; Pomroy *et al*., 1980; Buchet *et al*., 1981b; a). Approximately 75 percent of the excreted arsenic is methylated, about one-third as MMA and two-thirds as DMA.

The possibility of genetic polymorphism in arsenic metabolism has been suggested by Vahter *et al*. (1995a), who studied native Andean women in northwestern Argentina who were exposed to a wide range of As concentrations in drinking water (2.5 to 200 μg As/L). The women exposed to the highest As concentration in water exhibited surprisingly low levels of MMA in their urine (2.3 percent of metabolites). The percentage of arsenic urinary metabolites as MMA in typical human urine ranges from 12 to 20. Chiou *et al*. (1997a) studied the relationships among arsenic methylation capacity, body retention, and genetic polymorphisms of glutathione-*S*-transferase (GST) M1 and T1 in 115 human subjects. Percentages of As species in urine (mean ± SE) were: Asi, 11.8 ± 1.0; MMA, 26.9 ± 1.2; and DMA, 61.3 ± 1.4. Genetic polymorphisms of GST M1 and T1 were significantly associated with As methylation. Subjects with the null genotype of GST M1 had an increased percentage of Asi in urine, while those with the null genotype GST T1 had elevated DMA in their urine samples.

Marnell *et al*. (2003) reported six polymorphisms in the MMA^V reductase hGSTO1 gene in DNA isolated from peripheral blood of 75 Mexican subjects. Two subjects with the same polymorphism showed 5 to 10 fold higher concentrations (μg/g creatinine) of Asi in their urine than other subjects.

Yu *et al*. (2003) screened DNA of 22 subjects of European ancestry (EA) and 24 of indigenous American ancestry (IA) for polymorphisms in arsenate reductase and MMA^V reductase genes. For the arsenate reductase gene (hPNP) 48 polymorphic sites were identified while 33 were found in the MMA^V reductase gene (hGSTO1-1). For the EA individuals the MMA^V reductase gene showed greater polymorphism than the arsenate reductase gene whereas the reverse was seen in the IA individuals. In the latter group only one polymorphism had a frequency of > 10%. Meza *et al*. (2005) screened 135 As-exposed subjects from Sonora, Mexico for polymorphisms in arsenic metabolism genes: arsenate reductase (hPNP); MMA^V reductase (hGSTO); and arsenic 3 methyltransferase (CYT19). The subjects were exposed to drinking water with 5.5 to 43.3 ppb arsenic. The screening was based on urinary DMA^V/MMA^V (D/M) ratios. The analysis revealed that all of the variation was due to a very strong association between CYT19 and D/M in children only (7-11 yr). With children removed no significant association was seen in adults (18-79 yr). This developmentally regulated association between CYT19 and arsenic metabolism raises questions about the adequacy of arsenic risk assessment for children.

Several authors have studied the kinetics of As excretion in humans. Tam *et al*. (1979) administered ^{74}As arsenic acid (0.01 μg, ca. 6 μCi) to six adult males (age: 28-60; body weight: 64-84 kg) following an overnight fast. The urine was analyzed at 24 hr intervals for five days following As administration. In the first 24 hr period Asi excretion exceeded that of the methylated metabolites but thereafter the usual DMA > MMA > Asi pattern persisted, with DMA increasing in percentage of cumulative excretion at the later time points. A follow up study (Pomroy *et al*., 1980) followed ^{74}As excretion for periods up to 103 days using a whole body counter, with measurement of excreta for the first seven days. Their results indicate that the excretion data were best represented by a three-component exponential function. The coefficients for the pooled data accounted for 65.7 percent of excretion with a half-life of 2.09 days, 30.4 percent with a half-life of 9.5 days, and 3.7 percent with a half-life of 38.4 days. A four-exponent function showed a better fit to one of the six subjects (half-lives: 0.017, 1.42, 7.70 and 44.1 days).

Physiologically-based pharmacokinetic (PBPK) models employ data from various sources to mathematically simulate the uptake, distribution, metabolism and excretion of toxic chemicals in species of interest. Such models are used in risk assessment to estimate target tissue doses and to facilitate route-to-route and interspecies extrapolations. By contrast, pharmacodynamic (PD) models simulate biological responses to chemical exposures. A number of PBPK models for arsenic disposition and metabolism have been developed for experimental animals and humans (Mann *et al*., 1994; Menzel *et al*., 1994; Mann *et al*., 1996a; 1996b; Yu, 1999; Gentry *et al*., 2004). Although these models are based on somewhat different principles, they all seem to do a fair job in predicting the overall disposition of arsenic in animals and man. However, while the models often incorporate the latest ideas on the metabolism of inorganic arsenic with respect to oxidation state, methylated metabolites, and enzyme inhibition, due to limitations in our understanding of the modes of action of arsenic toxicity, they have yet to include representations of biological responses or pharmacodynamic (PD) capabilities, such as dosimetry linked alterations of DNA methylation, cell signaling pathways, DNA repair inhibition or generation of reactive oxygen species.

As an example of the complexity of arsenic action, Gentry *et al*. (2004) observed that pharmacodynamic changes occurred in mice without changes in PBPK predicted arsenic tissue

dosimetry. These authors used the PBPK model of Mann *et al*. (1996a,b) extended to mice to evaluate possible dosimetry differences between mouse a strain susceptible to arsenic induced tumors (C57Bl/6J) and those that lacked susceptibility (e.g., Swiss CD-1, Swiss CD: NIH(S), C57Bl/6p53 (+/-)). The model was parameterized using published acute mouse data for arsenate, arsenite, MMA and DMA and validated with acute exposure data from the C57Black mouse strain. Model predictions for acute exposure were then compared with data from acute (24 hr) and chronic exposures (26 weeks). No differences were seen in the volume of distribution or tissue-plasma concentration ratios between acute and chronic exposures. Comparison of metabolite profiles in blood, liver and urine also showed little difference between acute and chronic exposures. Model predictions compared well with observed values. The authors concluded "… that pharmacokinetic factors do not provide an explanation for the difference in outcomes across the various mouse bioassays." This conclusion may be overly broad since all the metabolites of arsenic and its metabolic pathways were not included in the PBPK modeling.

Liao et al. (2008) employed PBPK models with age-specific parameters to estimate urinary excretion of methylated arsenic metabolites in children. The results were coupled with skin lesion data from West Bengal, Bangladesh and Taiwan to derive dose-response relationships based on MMA^{III} in urine and concentration and duration of exposure to inorganic arsenic in drinking water using the Weibull (dose and time) model. While MMA^{III} was not specifically modeled a ratio of 7.4/2.8% MMA^{III}/MMA^{V} in total urinary MMA excretion was assumed. Age-specific risks at the $ED_{0.1}$ level (10^{-3} risk) were calculated for 0 -<1, 1-6, 7-12, and 13-18 yr age groups. Hyperpigmentation was a more sensitive endpoint than keratosis and males gave lower $ED_{0.1}$ values than females with values of 2.82, 1.51, 1.08, and 0.91 μg As/L for hyperpigmentation in males in the respective age groups. Age specific median daily drinking water consumption rates of 0.65, 1.29, 1.75, and 2.22 L/d, respectively, were used. Although the authors claim these concentrations as "Recommended Safe" levels, they are specific for 1/1000 risk and the skin lesion endpoint, which is not the most sensitive adverse effect for arsenic in exposed children.

4.2 Arsine

Although most studies of arsenic metabolism have centered on arsenate and arsenite, other forms of arsenic are also metabolized in humans. Apostoli *et al*. (1997) reported on the metabolism of arsine gas ($As^{-III}H_3$) in an occupationally exposed worker. Arsenic species were analyzed in urine over a five-day post-exposure period by liquid chromatography and inductively coupled plasma mass spectroscopy. The As species most excreted were MMA, DMA, As^{III}, arsenobetaine (AsB), and to a lesser extent As^{V}. The data indicate a capability to oxidize As^{-III} to As^{V} species probably via arsenite As $(OH)_3$. Arsenobetaine, an important form of arsenic in food, does not undergo subsequent biotransformation and is excreted via the urine. Curiously, arsenobetaine does not appear to be a metabolite of arsine in rats exposed for 1 hour to 4 to 80 mg/m^3 arsine (Buchet *et al*., 1998). The apparent similarity of the metabolism of arsine and arsenite is important and supports the use of the inorganic arsenic RELs for arsine.

Carter *et al*. (2003) have reviewed the metabolism of arsenic oxides, gallium arsenide and arsine. These authors describe three reactions that appear to occur in aqueous solutions of arsine (-III): (1) the formation of elemental As^0 and hydrogen; (2) reaction of AsH_3 with oxidized thiols to form diarsine AsH_2-AsH_2 (proposed) and reduced thiol RSH; and (3) possible reaction between

arsine and oxygen species, producing arsine hydroperoxide H_2AsOOH (Hatlelid *et al*., 1995; 1996). Relatively few studies of arsine metabolism have been conducted in experimental animals. In vitro studies indicate that arsine was rapidly distributed to red blood cells. In plasma arsine appeared to decompose over a few hours. Arsine apparently undergoes rapid oxidative metabolism although the intermediary metabolites have not been identified and apparently are not identical with those shown above for arsenite metabolism (Scheme 1) (Carter *et al*., 2003). A hypothetical scheme based on the same alternate application of oxidative methylation and reduction steps might look as follows with double arrows indicating four electron oxidation steps and single arrows two electron reduction steps:

$$\mathbf{As^{-III}H_3 \rightarrow\!\!\!\rightarrow H_2As^{I}(O)CH_3 \rightarrow H_2As^{-I}CH_3 \rightarrow\!\!\!\rightarrow HAs^{III}(O)(CH_3)_2 \rightarrow HAs^{I}(CH_3)_2 \rightarrow\!\!\!\rightarrow As^{V}(O)(CH_3)_3}$$

Arsine **TMAO**

According to this scheme the intermediary metabolites would include methylated arsine and arsine oxide species. Alternatively nonmethylative oxidation of arsine could lead to arsenite and arsenate via hydroxylated arsine species. Other metabolites possibly based on the oxidation of elemental As or arising via the postulated arsine hydroperoxide are also possible.

5. Acute Toxicity of Arsenic and Arsenic Compounds

5.1 Acute Toxicity to Adult Humans

The relative acute toxicity of arsenic compounds decreases as follows: arsine (As^{-III}) > organo-arsine derivatives > arsenites (As^{III}) > arsenoxides ($As^{II}I$) > arsenates (As^{V}) > pentavalent organic compounds (As^{V}) > arsonium metals (As^{I}) > metallic arsenic (As^{0}), where the Roman numeral indicates the oxidation state (HSDB, 1995).

Acute inhalation exposure may result in severe irritation of the mucous membranes of the upper and lower respiratory tract with symptoms of cough, dyspnea, and chest pain (Friberg *et al*., 1986). These may be followed by garlicky breath and gastrointestinal symptoms including vomiting and diarrhea (HSDB, 1995). Signs of acute poisoning are dermatitis, nasal mucosal irritation, laryngitis, mild bronchitis, and conjunctivitis (Friberg *et al*., 1986). The acute toxic symptoms of trivalent arsenic poisoning are due to severe inflammation of the mucous membranes and increased permeability of the capillaries (HSDB, 1995). Ingestion of 2 grams of As_2O_3 was fatal to an adult male (Levin-Scherz *et al*., 1987).

5.2 Acute Toxicity to Infants and Children

Relatively little data are available on acute toxicity of arsenic compounds to children. Childhood poisonings due to arsenic have been reported in the medical literature, often with little dosimetry. Campbell & Oates (1992) surveyed 200 child poisonings and found of the four deaths reported one was due to arsenic-containing weed killer (probably cacodylic acid). Alternatively, the use of arsenic trioxide in cancer chemotherapy seems well tolerated. George *et al*. (2004) reported the treatment of 11 children with acute promyelocytic leukemia with i.v. 0.15 mg As_2O_3/kg-d (8 treatment cycles over a period of 12 months). The toxic effects noted, including leukocytosis and skin hyperpigmentation, were considered minimal. Relapse-free survival was 81%.

5.3 Acute Toxicity to Experimental Animals

The lethal concentration low (LC_{Lo}) for $AsCl_3$ in the cat for a 20-minute inhalation exposure is 100 ppm (740 mg/m³) (Flury, 1921). In the mouse, the LC_{Lo} of $AsCl_3$ for a 10-minute exposure is 338 ppm (2500 mg/m³) (Flury, 1931).

A single intratracheal instillation of 17 mg As_2O_3/kg in rats resulted in multifocal interstitial pneumonia and focal proliferative bronchiolitis and alveolitis observed at necropsy 14 days post-exposure (Webb *et al*., 1986). The authors suggest that As_2O_3 induced an acute fibrogenic response.

Changes in host resistance from inhalation exposure to As_2O_3 aerosol were examined in female CD1 mice using a streptococcus infectivity model and an assay for pulmonary bactericidal activity (Aranyi *et al*., 1981; Aranyi *et al*., 1985). Mice (100-200/group) were exposed to As_2O_3 aerosol (or filtered air) for 3 hours/day, 5 days/week, for 1, 5 or 20 days. Aerosol exposed and control mice were then combined before challenge with *Streptococcus zoopidemicus* aerosol (4-8 replicate exposures). Statistically significant increases in mortality ($P < 0.05$) were observed in mice exposed: (1) once to 271, 496, and 940 μg As/m^3; (2) 5 times to 519 μg As/m^3; and (3) 20 times to 505 μg As/m^3. Multiple exposures at a given exposure level did not correlate with increased mortality, suggesting an adaptation mechanism. Single exposures did, however, show a dose-response for increased mortality with increasing level of arsenic exposure. Bactericidal activity was evaluated by measuring the ratio of viable bacteria count to radioactive count in the lung 3 hours after infection with ^{35}S-labeled *Klebsiella pneumoniae*. A single exposure to 271, 496, and 940 μg As/m^3, but not 123 μg As/m^3, resulted in significantly decreased bactericidal activity. Five exposures to 519 μg As/m^3 and twenty exposures to both 245 and 505 μg As/m^3 resulted in decreased bactericidal activity. The studies indicate a NOAEL for immunotoxicity of 123 μg As/m^3. This study provides a partial mode of action of arsenic-induced increase in mortality due to experimental lung infections with the mouse pathogen *S. zooepidemicus*. The second bactericidal assay with radiolabelled *K. pneumoniae* provides a plausible explanation, namely that arsenic exposure above 123 μg/m^3 inhibits normal immune bactericidal response in the lung.

Among the other adverse effects of inorganic arsenic noted in experimental animals, the most interesting and relevant to the 8-hour and chronic RELs are those on the brain and nervous system. These include changes in brain histology and conditioned reflexes, changes in locomotor activity, and decreased acetyl cholinesterase, GAD, and GABA levels in the hypothalamus, brain stem and cerebellum. Arsenic induced alterations of brain structure and function are consistent with the more subtle neuro-developmental effects seen in children exposed to inorganic arsenic at lower environmental levels.

5.4 Developmental and Reproductive Toxicity

Arsenic is listed under California Proposition 65 (Cal/EPA, Safe Drinking Water and Toxic Enforcement Act of 1986) as a developmental toxicant. The oxidation state of arsenic determines the teratogenic potential of its inorganic compounds; trivalent (III) arsenic compounds possess greater teratogenic potential than pentavalent (V) compounds. In hamsters, a single maternal intravenous injection of 20 mg/kg sodium arsenate (V) ($AsHNa_2O_4$) on gestation

day 8 was lethal to 44% of all embryos (Willhite and Ferm, 1984). A smaller dose (10 mg/kg) of sodium arsenite (As^{III}) ($AsHNaO_2$) administered in the same manner resulted in 90% embryonic lethality.

Fetal malformations, including exencephaly, resulted from an intravenous injection of $AsH_3Na_2O_4$ (As^{V}) into pregnant hamsters on gestation day eight (Ferm and Carpenter, 1968). The reproductive NOAEL in this experiment was 5 mg/kg. A significant reduction in fetal body weight, but no malformations were observed following a maternal dose of 5 mg/kg AsH_2NaO_3 (As^{III}) by the same route on gestation day eleven or twelve (Harrison and Hood, 1981).

A significant increase in pre-implantation mortality followed exposure of pregnant rats to aerosolized As_2O_3 at 1 mg/m³ for 5 months; no maternal toxicity was observed (Kamkin, 1982). At the LOAEL, 0.3 mg/m³, slightly elevated pre-implantation lethality was observed. The validity of this report cannot be evaluated, however, because key experimental details were not reported

A significant decrease in spermatozoa motility was observed in male rats following continuous exposure to As_2O_3 at a concentration of 40 mg/m³ for 48 hours (Kamil'dzhanov, 1982). Intravenous injection of radioactive arsenate (As^{V}) or arsenite (As^{III}) in several rodent species, including mice and hamsters, resulted in accumulation of arsenic in the lumen of the epididymal duct, which suggests that long term exposure of sperm may occur *in vivo* following acute exposure to As (Danielsson *et al*., 1984).

Nagymajtenyi *et al*., (1985) exposed pregnant CFLP mice (8-11 females/group) to As_2O_3 aerosol for 4 hours/day on gestational days 9-12 at concentrations of 0, 0.26, 2.9, or 28.5 mg As_2O_3/m^3 (~0.2, 2.2, and 21.6 mg As/m^3. The aerosol was generated by spraying an aqueous solution of As_2O_3. On the 18th day of gestation the mice were sacrificed and the fetuses removed. The numbers of live and dead fetuses were recorded, weighed, and examined microscopically. Fifty fetuses were stained with Alizarin red-S for skeletal examination. Chromosome preparations were made from livers of 10 fetuses per exposure group. Twenty mitoses in each fetus (200/group) were scored for chromosomal damage and 10 percent of these were karyotyped. The data were analyzed with either Fisher's exact test or in the case of fetal weights with the Dunnett multiple comparison t-test.

A statistically significant decrease in fetal weight was observed in all of the dose groups ($P < 0.05$), with a 3, 9, and 29% reduction in average fetal weight with increasing dose (Table 6.4.1). Significantly delayed bone maturation (ossification defects) was observed only in the highest dose group (sternum 14/50; limbs 32/50, both $p < 0.05$). However, an apparent positive dose-related trend in the number of fetuses with skeletal malformations was observed (2 [control], 3, 7, 31, respectively). A similar dose-related trend in chromosome aberrations in liver cells was also observed in the number of cells with damage (6[control], 10, 13, 24), chromatid gaps, chromatid breaks, chromosome fragments, and chromosome breaks (5[control], 10, 13, 27). Only the number of damaged cells and chromosome breaks at the high dose were significantly different from the control ($p < 0.05$).

Table 6.4.1 Data from Table 1 of Nagymajtényi et al. (1985).

As_2O_3 (mg/m^3)	Number of litters	Living fetuses per mother	Number of fetuses examined	% dead fetuses	Average fetal weight (grams)
28.5±0.3	11	9.6	100	29	0.981±0.04*
2.9±0.04	8	12.8.	100	13	1.146±0.03*
0.26±0.01	8	12.5	100	12	1.225±0.03*
0	8	12.5	100	8	1.272±0.02

* Significantly different from control ($p<0.05$)

This study demonstrates that inhalation exposure to inorganic arsenic is markedly fetotoxic. Arsenic concentrations of 28.5 mg/m^3 caused a reduction in the number of live fetuses, in fetal weight, and an increase in fetuses with delayed osteogenesis.

Rats exposed to 1 μg As_2O_3/m^3 (0.76 μg As/m^3) for 5 months showed increased preimplantation mortality and delayed ossification in fetuses (Kamkin, 1982). Experimental detail was not presented, thus limiting the usefulness of this study.

A significant decrease in spermatozoa motility was observed in male rats following continuous exposure to 32.4 mg As_2O_3/m^3 for 48 hours (Kamil'dzhanov, 1982). Similarly, motility was decreased after: (1) a 120-hour exposure to 7.95 mg/m^3; (2) a 252-hour exposure to 1.45 mg/m^3; and (3) an 800-hour exposure to 0.36 mg/m^3.

Holson *et al*. (1999) administered arsenic trioxide (As_2O_3) by whole body inhalation to groups of 25 Crl:CD (SD)BR female rats every day for six hours per day, beginning fourteen days prior to mating and continuing throughout mating. The target exposure levels were 0.3, 3.0, and 10.0 mg As_2O_3/m^3 (measured means: 0.24, 2.6, 8.3 mg As/m^3). Maternal toxicity evidenced by the occurrence of rales, a decrease in net body weight gain, and decreased food intake during pre-mating and gestation exposure, was observed only at the high dose. The NOAEL for maternal toxicity was 2.6 mg As/m^3 (3.4 mg As_2O_3/m^3). No treatment-related malformations or developmental variations were observed at any exposure level. The NOAEL for developmental toxicity was 8.3 mg As/m^3 (11 mg As_2O_3/m^3). The median mass aerodynamic diameter of particle sizes generated in the exposure chambers ranged from 1.9 to 2.2 μm for the three doses indicating that the dusts were respirable. However there were no blood or urine arsenic analytical data to assess delivered doses.

Nemec *et al*. (1998) evaluated the developmental toxicity of inorganic arsenic in mice and rabbits. CD-1 mice (25/dose group) and New Zealand White rabbits (20/dose group) were gavaged with aqueous arsenic acid (H_3AsO_4) doses of 0, 7.5, 24, or 48 mg/kg-d on gestation days (GD) six through 15 (mice) or 0, 0.19, 0.75, or 3.0 mg/kg-d on GD six through 18 (rabbits). The

animals were examined at necropsy (GD 18, mice; GD 29, rabbits). Treatment related maternal toxicity including mortality (2/25) was observed only in the highest dose administered to mice. Effects on maternal weight gain were noted only on GD 6-9 ($P < 0.01$) and GD 15-18 ($P < 0.05$) of the mid dose and on GD 6-9 ($p < 0.05$) of the low dose. While overall maternal weight gains were statistically significantly reduced only at the top dose there was an apparent negative trend in decreased GD18 body weights with increasing dose (56.2 g control, 54.9 g, 52.7g, 46.7g, respectively). While the authors identified a NOAEL for maternal toxicity of 7.5 mg/kg-d, the apparent negative trend noted above suggests that this may be a LOAEL (4.0 mg As/kg-d).

Statistically significant adverse effects on offspring growth or survival were seen only at the highest dose of 48 mg/kg-d. However, there was an apparent negative trend in the number of live fetuses per litter with increasing dose (12.3 control, 11.6, 11.0, 6.6, respectively). An increased incidence of resorptions per litter was seen in the 48 mg/kg-d dose group ($P \leq 0.01$), (mainly early resorptions). Early and total resorptions showed an apparent positive trend (6.4% total control, 6.1%, 9.6%, 41.9%, respectively). Mean fetal weight showed an apparent negative trend (1.3 g control, 1.32 g, 1.23 g, 0.99 g, respectively). There were no statistically significant dose-related increases in the overall incidence of fetal malformations; however, the mean percent of litter malformation was about three-fold higher in the 48 mg/kg-d dose group than in the lower doses and control. The NOAEL for developmental toxicity would appear to be 7.5 mg/kg-d (4.0 mg As/kg-d).

Maternal toxicity in rabbits, including mortality, slight body weight loss, and clinical signs (decreased urination and defecation, occasional prostration and ataxia), occurred only at the high arsenic acid dose of 3.0 mg/kg-d. The number of does with decreased urination and defecation appeared to be slightly higher in the mid- and low-dose groups, but these effects may not have been treatment related and no effects on body weight were seen. At sacrifice on GD 29 maternal body weight appeared to be reduced in the high dose group. A significant loss in mean maternal gravid body weight occurred during the first six days of high-dose treatment (GD 6-12) ($p \leq 0.01$). This effect persisted and was significantly different from controls for the entire treatment interval (GD 6-18). There were no statistically significant increases in the incidences of any developmental parameters, including malformations. Fetal survival, mean fetal weight, and sex ratio on GD 29 were not affected by the treatment. The number of live fetuses per litter was reduced and resorptions per litter increased in the high-dose group. The latter findings were mainly due to one doe with a totally resorbed litter. The overall values were the range from laboratory historical controls. The authors identified a NOAEL of 0.75 mg/kg-d (0.4 mg As/kg-d) for both maternal toxicity and developmental toxicity.

Stump *et al*. (1999) administered either sodium arsenate (As^{V}) i.p. or arsenic trioxide (As^{III}) i.p. or by gavage on GD 9 to 25 Crl:CD (SD) BR rats. The doses of sodium arsenate were 0, 5, 10, 20, and 35 mg/kg (0, 1.2, 2.4, 4.8, 8.4 mg As/kg). The doses of arsenic trioxide were: i.p. 0, 1, 5, 10, and 15 mg/kg (0, 0.8, 3.8, 7.6, and 11.4 mg As/kg); and by gavage (p.o.) 0, 5, 10, 20, 30 mg/kg (0, 3.8, 7.6, 15.2, 22.7 mg As/kg). Sodium arsenate (i.p.) caused decreased maternal food consumption (GD 9-20), decreased body weights and body weight gains at the highest dose of 35 mg/kg. Decreased food consumption was also seen in the 20 mg/kg dose group at GD 9-10 and GD 9-20. Arsenic trioxide (i.p.) resulted in excessive mortality in the highest dose-group (19/25) and significant reductions in maternal food consumption, body weight at GD20, body weight change, and net body weight in the next highest dose-group (10 mg/kg). Arsenic trioxide (p.o.)

resulted in less mortality in the highest dose-group (7/25). Clinical signs were noted in the 20 and 30 mg/kg dose-groups including changes in fecal consistency and decreased defecation. Food consumption (GD 9-10) was decreased in a dose-dependent manner across As treatment groups. The study identified single dose maternal effects NOAELs of 2.4 mg As/kg for sodium arsenate (i.p.) and 3.8 mg As/kg for arsenic trioxide i.p. A LOAEL of 3.8 mg As/kg was identified for arsenic trioxide p.o.

Intraperitoneal administration of sodium arsenate or arsenic trioxide caused neural tube and ocular defects (exencephaly, microphthalmia/anophthalmia, and other craniofacial defects) in the offspring of treated rats. These effects were statistically significant only at doses causing maternal toxicity or mortality (35 and 10 mg/kg, respectively). Oral administration of arsenic trioxide caused no treatment-related malformations. The study identified single dose developmental NOAELs of 2.4 mg As/kg for sodium arsenate i.p., 3.8 mg As/kg for arsenic trioxide i.p., and 15.2 mg As/kg for arsenic trioxide p.o.

DeSesso *et al*. (1998), in a comprehensive review of the developmental toxicity of inorganic arsenic, concluded that cranial neural tube defects (NTDs) were induced in rodents only when exposure occurred early in gestation, at high maternally toxic doses, and by parenteral routes of administration. They argued that such NTD effective doses are unlikely to be achieved by the oral, inhalation, or dermal routes in rodents, and that inorganic arsenic does not represent a realistic developmental risk in humans subjected to any environmentally relevant exposure scenarios.

Male and female Charles River CD mice (10/group) were treated with 0 or 5 ppm arsenite in drinking water continuously through three generations (Schroeder and Mitchener, 1971). Endpoints examined included the interval between litters, the age at first litter, the ratio of males to females, the number of runts, stillborn offspring, failures to breed, and congenital abnormalities. The study showed an alteration in the number of small litters in the arsenic exposed group.

Female CD-1 mice (8-15/group) were treated by oral gavage with 0, 20, 40, or 45 mg sodium arsenite/kg on a single day of gestation between days 8 and 15 (Baxley *et al*., 1981). Maternal mortality, fetal malformations, and increased prenatal death were observed among animals treated with 40 and 45 mg sodium arsenite/kg.

Pregnant golden hamsters (>10/group) were treated by oral gavage with a single administration of 0, 20, or 25 mg/kg sodium arsenite on one of gestational days 8-12 (Hood and Harrison, 1982). Prenatal mortality was increased among animals receiving 25 mg/kg on gestational days 8 and 12 and fetal weights were decreased among animals receiving 25 mg/kg on gestational day 12. One dam died following administration of 20 mg/kg.

Intravenous injection of radioactive arsenate (V) or arsenite (III) in several rodent species, including mice and hamsters, resulted in accumulation of arsenic in the lumen of the epididymal duct, which suggested that long term exposure of sperm to arsenic may occur *in vivo* following acute exposure (Danielsson *et al*., 1984).

6. Chronic Toxicity of Arsenic and Arsenic Compounds

6.1 Chronic Toxicity to Adult Humans

Arsenic in drinking water is carcinogenic to humans (Group 1, IARC, 2004). Arsenic compounds show limited to sufficient evidence of carcinogenicity in experimental animals (IARC, 2004). The U.S. Environmental Protection Agency has classified arsenic as Group A; a human carcinogen, based on sufficient evidence from human data including increased lung cancer mortality in multiple human populations exposed primarily through inhalation, increased mortality from multiple internal organ cancers (liver, kidney, lung, bladder), and increased skin cancers observed in populations exposed to arsenic in drinking water (IRIS online file www.epa.gov/iris/subst/0278.htm). Since this document deals with noncancer risks, the carcinogenicity of arsenic is not covered here in any detail (see OEHHA (1999)).

Smelter workers, exposed to concentrations of arsenic up to 7 mg As/m^3, showed an increased incidence in nasal septal perforation, rhinopharyngolaryngitis, tracheobronchitis, and pulmonary insufficiency (Lundgren, 1954).

In a case-control study, copper smelter workers (n = 47) exposed to arsenic for 8-40 years (plus 50 unexposed controls matched for age, medical history, and occupation) were examined by electromyography and for nerve conduction velocity in the arms and legs (Blom *et al*., 1985). The workers were found to have a statistically significant correlation between cumulative exposure to arsenic and reduced nerve conduction velocities in three peripheral nerves (upper and lower extremities). Slightly reduced nerve conduction velocity in 2 or more peripheral nerves was reported as "more common" among arsenic exposed workers. Minor neurological and electromyographic abnormalities were also found among exposed workers. Occupational exposure levels were estimated to be 0.05-0.5 mg As/m^3, with As_2O_3 the predominant chemical form. Except for three arsenic exposed workers who had long-term exposure to lead, exposure to other heavy metals was insignificant.

The smelter workers described by Blom *et al*. (1985) (number of controls reduced to 48) were further examined for prevalence of Raynaud's phenomenon and for vasospastic tendency by measurement of finger systolic pressure at 10°C and/or 15°C relative to that at 30°C (FSP%) (Lagerkvist *et al*., 1986). The FSP% was found to covary with the duration of exposure to arsenic, and the prevalence of Raynaud's phenomenon was significantly increased among exposed workers. Daily arsenic uptake was estimated at less than 300 µg/day and was confirmed with urinary excretion data.

Hyperpigmentation and hyperkeratinization were observed in workers exposed to 0.4 - 1 mg/m^3 inorganic arsenic for two or more years (Perry *et al*., 1948).

Most of the relevant epidemiological data on arsenic adverse effects comes from studies of arsenic exposure via drinking water. These studies are relevant because arsenic exerts similar toxic effects once it enters the body. For example, arsenic causes lung cancer in humans by both oral and inhalation routes. The adverse effects summarized below include skin lesions(keratosis and altered pigmentation), vascular effects on the heart, brain and peripheral vasculature, peripheral neuropathy, and lung disease.

6.1.2.1 Skin Effects

Mazumder *et al*. (1998) investigated arsenic-associated skin lesions of keratosis and hyperpigmentation in 7683 exposed subjects in West Bengal, India. While water arsenic concentrations ranged up to 3400 μg/L, over 80% of the subjects were consuming water with < 500 μg/L. The age-adjusted prevalence of keratosis was strongly related to water As concentration, rising from zero in the lowest exposure level (< 50 μg/L) to 8.3% for females drinking water containing >800 μg As/L, and from 0.2 to 10.7% in males, respectively. A similar dose-response was observed for hyperpigmentation: 0.3 to 11.5% for females; and 0.4 to 22.7% for males. Overall males had 2-3 times the prevalence of both keratosis and hyperpigmentation than females apparently ingesting the same doses of arsenic per body weight. Subjects that were more than 20% below standard body weight for their age and sex had a 1.6-fold increase in the prevalence of keratoses, suggesting that malnutrition may play a role in increasing susceptibility.

Rahman *et al*. (2006) evaluated arsenic exposure and age- and sex-specific risk for skin lesions in a population-based case-referent study in Bangladesh. The entire population over four years of age of Matlab, Bangladesh (N = 166,934) was screened for skin lesions. Skin lesions were classified as hyperpigmentation (melanosis), hypopigmentation (leukomelanosis), or keratosis. A total of 504 cases with skin lesions were identified. A randomly selected referent group of 1830 subjects was included in the study. Arsenic exposure was assessed by personal history of tube well use since 1970 or year of birth if later. Water samples from all functioning tube wells were measured for arsenic concentration by hydride-generation atomic absorption spectroscopy. A dose-response relationship was observed for increased skin lesions and arsenic exposure for both sexes ($P < 0.001$). For males using the metric of As μg/L the highest exposure quintile (≥ 300 μg/L) gave an adjusted odds ratio (OR) of 9.56 (95% CI = 4.20-21.8). Females gave a corresponding OR of 6.08 (3.06-15.5). The cumulative As exposure metric (μg/L x years) gave OR's of 10.4 and 9.19, respectively. In an analysis with males and females combined, adjusted for age and socioeconomic status, males had significantly higher risk of As-related skin lesions than females, when females' lowest average exposure quintile was used as the reference. For the highest quintile, the males OR was 10.9 (5.8-20.4) and the females OR was 5.78 (3.10-10.8), $P = 0.005$.

Dermatitis and irritation of the mucous membranes have been observed in arsenic-exposed workers (Vallee *et al*., 1960). Hepatic fatty infiltration, central necrosis, and cirrhosis were observed in two patients who ingested As_2O_3 (1% in Fowler's solution) for three or more years (Morris *et al*., 1974). Daily consumption of 0.13 mg As/kg in contaminated well water resulted in the chronic poisoning and death of four children; at autopsy, myocardial infarction and arterial thickening were noted (Zaldivar and Guillier, 1977).

6.1.2.2 Vascular Disease

Vascular diseases have long been noted to be associated with chronic arsenic exposures among German vineyard workers (Grobe, 1976) and inhabitants of Antofagasta, Chile (Borgono *et al*., 1977). Peripheral vascular diseases have been reported to be associated with the occurrence of arsenic in well waters in Taiwan (Chen and Wu, 1962; Chi and Blackwell, 1968; Tseng, 1977; Chen *et al*., 1988). Concentrations in one study were characterized as 0.10 – 1.8 ppm (Yu *et al*.,

1984). The term arseniasis or arsenosis connotes vascular disease associated with chronic exposure to arsenic, specifically blackfoot disease (BFD). BFD is characterized by progressive narrowing of the peripheral arteries, particularly those of the lower extremities. This can lead to ulceration, gangrene and amputation. The etiology of BFD is unclear but arsenic is thought to be the principal cause. The term arsenicosis refers to arsenic induced skin lesions ranging in severity over four stages, seven grades and 20 sub-grades from diffuse melanosis (skin pigmentation or depigmentation) to aggressive skin and internal malignancy (Saha, 2003).

Wu *et al*. (1989) found significant trends of mortality rates from peripheral vascular diseases and cardiovascular diseases with concentrations of arsenic in well water. However, no significant association was observed for cerebrovascular accidents. Engel and Smith (1994) evaluated arsenic in drinking water and mortality from vascular disease in 30 U.S. counties from 1968 to 1984. Mean As levels in drinking water ranged from 5.4 to 91.5 μg/L. Standardized mortality ratios (SMRs) for diseases of arteries, arterioles, and capillaries (DAAC) for counties exceeding 20 μg/L were 1.9 (90% C.I. = 1.7-2.1) for females and 1.6 (90% C.I. = 1.5-1.8) for males. SMRs for three subgroups of DAAC including arteriosclerosis and aortic aneurysm were also elevated as were congenital abnormalities of the heart and circulatory system.

Tseng *et al*. (1996) studied the dose relationship between peripheral vascular disease (PVD) and ingested inorganic arsenic in blackfoot disease endemic villages in Taiwan. A total of 582 adults (263 men and 319 women) underwent Doppler ultrasound measurement of systolic pressures on bilateral ankle and brachial arteries and estimation of long-term arsenic exposure. The diagnosis of PVD was based on an ankle-brachial index of < 0.9 on either side. Multiple logistic regression analysis was used to assess the association between PVD and As exposure. A dose-response relationship was observed between the prevalence of PVD and long-term As exposure. The odds ratios (95% confidence intervals) after adjustment for age, sex, body mass index, cigarette smoking, serum cholesterol and triglyceride levels, diabetes mellitus and hypertension were 2.77 (0.84-9.14), and 4.28 (1.26-14.54) for those who had cumulative As exposures of 0.1 to 19.9 and ≥ 20 (mg/L) x yr, respectively. A follow up study (Tseng *et al*., 1997) indicated that PVD was correlated with ingested As and not with abnormal lipid profiles. The lipid profiles studied were total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c), apolipoprotein AI, and apolipoprotein B. Other lipids such as modified LDL, subclasses of LDL and HDL, and other lipoproteins such as lipoprotein (a), which may track as better indicators of atherosclerosis, were not included. Also, the roles of platelet aggregation and coagulation profiles were not studied.

Chen *et al*. (1996) evaluated the dose-response relationship between ischemic heart disease (ISHD) mortality and long-term arsenic exposure. Mortality rates from ISHD among residents in 60 villages in an area of Taiwan with endemic arseniasis from 1973 through 1986 were analyzed for association with As concentrations in drinking water. Based on 1,355,915 person-years and 217 ISHD deaths, the cumulative ISHD mortalities from birth to age 79 yr were 3.4%, 3.5%, 4.7%, and 6.6% for the median As concentrations of < 0.1, 0.1-0.34, 0.35-0.59, and ≥ 0.6 mg/L, respectively. Multivariate-adjusted relative risks (RRs (95% C.I.)) associated with cumulative arsenic exposure from well water were 2.46 (0.53-11.36), 3.97 (1.01-15.59), and 6.47 (1.88-22.24) for 0.1-9.9, 10.0-19.9, and 20+ (mg/L)-yr, respectively, compared with those without As exposure.

Chiou *et al*. (1997b) evaluated the dose-response relationship between prevalence of cerebrovascular disease and ingested arsenic among residents of the Lanyang Basin in northeast Taiwan. A total of 8102 adults from 3901 households were recruited for the study. Arsenic in well water of each household was determined by hydride generation and atomic absorption spectrometry. Logistic regression analysis was used to estimate multivariate-adjusted odds ratios and 95% confidence intervals for various risk factors of cerebrovascular disease. A significant dose-response relationship was observed between As concentration in well water and prevalence of cerebrovascular disease after adjustment for age, sex, hypertension, diabetes mellitus, cigarette smoking, and alcohol consumption. The dose-response relationship was even more prominent for cerebral infarction with multivariate-adjusted odds ratios (95% C.I.) of 1.0, 3.4 (1.6-7.3), 4.5 (2.0-9.9), and 6.9 (3.0-16), respectively, for those who consumed well water with As concentrations of 0, 0.1-50.0, 50.1-299.9, and > 300 μg/L. For cumulative arsenic exposures of <0.1, 0.1-4.9, and ≥ 5.0 (mg/L)-yr, the odds ratios were 1.00, 2.26, and 2.69 for cerebrovascular disease and 1.00, 2.66, and 3.39 for cerebral infarction, respectively. All of the values above for As exposed groups were significantly greater than unexposed at $P < 0.05$.

Chen *et al*. (1995) also investigated the association between long-term exposure to inorganic arsenic and the prevalence of hypertension. A total of 382 men and 516 women were studied in villages where arseniasis was endemic. Hypertension was defined as a systolic blood pressure of 160 mm Hg or greater, or a history of hypertension treated with antihypertensive drugs. The long-term arsenic exposure was calculated from the history of artesian well water consumption obtained through subject questionnaires and the measured arsenic concentration in well water. Residents in villages where long-term arseniasis was endemic had a 1.5-fold increase in age- and sex-adjusted prevalence of hypertension compared with residents in nonendemic areas. The duration of well water consumption, average As water concentration, and cumulative As exposure were all significantly associated with hypertension. For the cumulative As exposure in (mg/L)-yr, the percent prevalence values were: 0, 5.0%; 0.1-6.3 (mg/L)-yr, 4.9%; 6.4-10.8 (mg/L)-yr, 12.8%; 10.9-14.7 (mg/L)-yr, 22.1%; 14.8-18.5 (mg/L)-yr, 26.5%; > 18.5 (mg/L)-yr, 29.2%.

As part of a study of arsenic exposure via drinking water and mortality outcome in Millard County, Utah, Lewis *et al*. (1999) found a statistically significant association with mortality from hypertensive heart disease. Median drinking water concentration of arsenic ranged from 14 to 166 μg/L for the 946 subjects in the study. The standard mortality ratios (SMR) without regard to specific exposure levels were SMR = 2.20 (95% C.I., 1.36-3.36) for males and SMR = 1.73 (95% C.I., 1.11-2.58) for females. When analyzed by cumulative exposure groups of low (< 1.0 (mg/L)-yr), medium (1.0-4.9 (mg/L)-yr), and high (≥ 5.0 (mg/L)-yr), there was no apparent dose response relationship. However the cumulative dose estimates in this study were lower than in the Chen *et al*. (1995) discussed above so the results of the two studies are not inconsistent.

Chen *et al*. (2006) conducted a cross-sectional analysis of the association of arsenic exposure from drinking water and blood pressure in 10,910 subjects. Time-weighted well arsenic concentrations (TWA) based on current and past well usage were derived. Odds ratios (OR's) for high pulse pressure (systolic – diastolic pressure ≥ 55 mmHg) by increasing TWA quintiles (≤ 8, 8.1-40.8, 40.9-91.0, 91.1-176.0, 176.1-864.0 μg/L) were: 1.00 (referent); 1.39 (95% C.I. 1.14, 1.71); 1.21 (0.9, 1.49); 1.19 (0.97, 1.45); 1.19 (0.97,1.46). OR's for systolic hypertension (≥ 140 mmHg) suggested a similar but weaker association. Participants with lower than average

intake of B vitamins and folate showed somewhat higher OR's. No associations were apparent for TWA and diastolic hypertension.

In a study related to those above, Lai *et al*. (1994) studied inorganic arsenic ingestion and the prevalence of diabetes mellitus. A total of 891 adult residents of villages in southern Taiwan where arseniasis is endemic were included in the study. Diabetes status was determined by an oral glucose tolerance test and a history of diabetes regularly treated with sulfonylurea or insulin. Cumulative arsenic exposure in ppm-yr was determined from the detailed history of drinking artesian well water. There was a dose-response relation between cumulative arsenic exposure and prevalence of diabetes mellitus. The relation remained significant after adjustment for age, sex, body mass index, and activity level at work by a multiple logistic regression analysis giving multivariate-adjusted odds ratios of 6.61 and 10.05, respectively, for exposures of 0.1-15 ppm-yr and > 15.0 ppm-yr versus an unexposed group. In an effort to confirm this association between diabetes mellitus and arsenic observed for drinking water in Taiwan, Rahman and Axelson (1995) reviewed 1978 case-control data from a Swedish copper smelter. Twelve cases of diabetes mellitus (death certificate) were compared with 31 controls without cancer, cardiovascular and cerebrovascular disease. The odds ratios for diabetes mellitus with increasing arsenic exposure categories were 1.0 (reference level), 2.0, 4.2, and 7.0 with the 95% confidence level including unity. The trend was weakly significant, $p = 0.03$. Albeit with limited numbers, the study provides some support for a role of arsenic exposure in the development of diabetes mellitus.

6.1.2.3 Neurological Disease

Hafeman *et al*. (2005) evaluated the association between arsenic exposure and peripheral neuropathy in a cross-sectional study of 137 adults in Bangladesh. Exposure measures included individual arsenic water concentration, cumulative arsenic index (CAI), and urinary arsenic concentration. Experimental measures were primarily vibrotactile threshold testing of the index finger (IVT) and toe (TVT) and secondarily tapping speed, grip strength, ankle reflex, and proprioception. The cumulative arsenic index and urinary arsenic were both significantly associated with elevated TVT ($P = 0.02$ and $P = 0.009$, respectively) after adjustment for age and gender. While dose-response relations were difficult to define, a linear regression analysis of TVT (vibration units) versus the continuous measures of urinary arsenic and CAI gave slopes of 0.02 and 0.0025 TVT units/50 μg As/mg urinary creatinine, respectively. The association between IVT and arsenic exposure was not statistically significant. No association was found between any measure of arsenic exposure and grip strength, tapping speed, ankle reflex, or proprioception.

6.1.2.4 Lung Disease

Several studies have reported effects of arsenic exposure through drinking water on the lung. Mazumder et al. (2000) reported increasing respiratory symptoms, including cough, shortness of breath, and chest sounds, with increasing arsenic concentrations in the drinking water in people residing in West Bengal, India. The effects seen were marked in individuals who also had arsenic related skin lesions. In a later study also in West Bengal, these investigators also reported a large increase (OR = 10; 95% CI 2.7-37) in bronchiectasis in individuals with skin lesions compared to those without arsenic-related skin lesions (Mazumder et al., 2005).

Von Ehrenstein *et al*. (2005) studied the relation between lung function, respiratory symptoms, and arsenic in drinking water among 287 adults, including 132 with arsenic-induced skin lesions in West Bengal, India. Arsenic levels in drinking water and the number of male subjects with or without skin lesions were: 0-99 μg/L, 9, 36; 100-399 μg/L, 66, 34; ≥400 μg/L, 18, 15, respectively. For respiratory symptoms of "shortness of breath at night" and "morning cough", the odds ratios (ORs) for men with skin lesion versus those without was 2.8 with 95% confidence intervals (C.I.) of (1.1, 7.6) and (1.2, 6.6), respectively. For men with skin lesions, the average forced expiratory volume in one second (FEV_1) was reduced by 256.2 mL (95% C.I.; 113.9, 398.4) $P < 0.001$. Average forced vital capacity (FVC) was reduced by 287.8 mL (95% C.I.; 134.9, 440.8) $P < 0.001$. In men a 100 μg/L increase in arsenic level was associated with a 45.0 mL decrease (95% C.I.; 6.2, 83.9) in FEV_1 ($P = 0.02$) and a 41.4 mL decrease (95% C.I.; -0.7, 83.5) in FVC ($P = 0.054$). The findings were adjusted for age, height and smoking in both males and females. Women participating in the study (N = 109) had a lower risk of developing skin lesions than men and exhibited few respiratory symptoms.

6.2 Chronic Toxicity to Infants and Children

The adverse effects of inorganic arsenic exposure reported in children include skin lesions, neurodevelopmental effects (IQ and related effects), lung disease expressed in later years, and reproductive effects (decreased birth weight, spontaneous abortion, neonatal death).

As noted above Mazumder *et al*. (1998) observed a dose-response for arsenic-associated skin lesions in a cross-sectional survey of 7683 subjects in West Bengal, India. The study population was divided by age decades such that the effect on young children (≤ 9 yr) and adolescents (10-19 yr) could be analyzed separately. The prevalence of keratosis in females and males was 0.2 and 0.5 percent in young children and 1.0 and 1.7 percent in adolescents, respectively. The comparable values for hyperpigmentation were 1.7 and 2.0 percent and 2.2 and 3.5 percent, respectively. Overall 1149 young children and 1599 adolescents were surveyed. The low- to mid-dose quantal responses for combined skin lesions in young children using the mid points of the arsenic concentration ranges (μg/L) were: 25, 0/414; 75, 0/95; 125, 4/118; 175, 2/50; 275, 6/161; 425, 11/101. For the adolescents the comparable values were: 1/730; 2/147; 2/107; 7/110; 26/213; 9/58.

The adverse effects of inorganic arsenic on the developing intellectual function of exposed children have been reported in several studies summarized in this section. While some of the studies have deficiencies, as a group they indicate that arsenic exposure, like lead exposure, presents a risk to children. The neurodevelopmental endpoint has been selected by OEHHA as the critical effect for deriving 8-hour and chronic RELs for inorganic arsenic.

Calderon *et al*. (2001) conducted a cross-sectional study to examine the effects of chronic exposure to lead (Pb) and arsenic (As), and also nutrition, on the neuropsychological development of children. Two populations of children aged six to nine years (N = 41, 39) with differing As exposure levels (63 vs. 40 μg/g) but similar Pb exposures (8.9 vs. 9.7 μg Pb/dL blood, respectively) were compared using the Wechsler Intelligence Scale for Children (WISC) Revised Version for Mexico. After controlling for significant potential confounders verbal IQ was observed to decrease with increasing urinary arsenic ($P < 0.01$). Language, verbal comprehension and long-term memory also appeared to be adversely affected by increasing

arsenic exposure (concepts and knowledge factors, $P < 0.05$ each). Blood lead was significantly associated with a decrease in attention (sequential factor, $P < 0.05$). However since blood lead is an imprecise measure of lead burden there could be some residual confounding in this study.

The relationship between arsenic exposure via drinking water and neurological development as indicated by intelligence (IQ) was assessed in Thailand (Siripitayakunkit *et al*., 1999) in 529 children aged six to nine years using a cross-sectional design. Arsenic levels in hair were used to assess exposure and the WISC test for children was used to assess IQ. The range of arsenic concentrations in hair was 0.48 to 26.94 μg/g (mean = 3.52, SD = 3.58). The mean IQ of the study was 90.44 (range 54 to 123). Most of the IQs were classified as average (45.7%) or dull normal (31.6%). Approximately 14% and 3% of the children were in the borderline and mental defective groups, respectively. The percentage of children in the average IQ group decreased significantly from 57 percent to 40 percent with increasing arsenic exposure. The percentage in the lower IQ group increased with increasing As (23% to 38%) and in the low IQ group (zero to six percent). In a comparison of IQ between children with As hair levels ≤ two ppm or > two ppm, arsenic was found to explain 14 percent of the variance in IQ after controlling for father's occupation, mother's intelligence score, and family income. Arsenic levels in hair above 2 ppm were associated with a 0.75-point decrease in IQ below the grand mean and As levels above 5 ppm with a two point decrease. Although the cross-sectional study design does not allow for establishment of the time precedence of exposure to arsenic, the investigators stated that the subjects of the study were born in a period of chronic arsenic poisoning and that this cohort has been continuously exposed since birth due to their non-mobility. The study suffers from small numbers of children exposed to low arsenic (hair arsenic ≤ 1 ppm) so this group could not be compared to the high arsenic children. Also the possible exposure to chemical confounders like lead was not discussed.

In a parallel cross-sectional study (Siripitayakunkit *et al.*, 2001) the 529 children (above) were subjected to the Motor-Free Visual Perception Test (MVPT) and the Visual-Motor Integration Test (VMI). The visual perception score of each child was compared with the score of children in a control sub-district of the same age. The cutoff point for poor perception was the mean minus one standard deviation (SD) in each age level. Among arsenic-exposed children, 21 percent had poor visual perception and 17.6 percent had poor VMI. The comparable values in the control population were 16.5 percent and 15.8 percent, respectively. Potential confounders were controlled by multiple classification analysis. Only five percent of the variance in visual perception of children was significantly explained by arsenic ($P = 0.01$). The grand mean perception score was 20.57 and the adjusted values at low, medium and high hair As were 20.92, 20.51, and 20.03, respectively. Alternatively, these authors did not find an effect of arsenic on visual-motor integration.

Like the study of IQ decrements noted above, this study has the advantage of associating an adverse effect in children with a metric of chronic arsenic exposure, hair arsenic concentration. Disadvantages include a limited level of reporting and possible confounding with exposure to other metals.

Tsai *et al*. (2003) performed a cross-sectional study of the effect of arsenic exposure on the development of cognitive function among adolescents. Forty-nine 13-year old students were divided into low and high exposure groups and were compared with 60 13-year old unexposed

children. Four neurobehavioral tests were conducted: continuous performance test (CPT); symbol digit (SD); pattern memory (PM); and switching attention (SA). Exposure in terms of As concentration in drinking water averaged 0 (<0.15), 131.2, and 185.0 ppb for control and exposure groups, respectively. Average cumulative arsenic exposures were 0, 252.1, and 768.2 mg (e.g., 184.99 ppb x 1008.6 cm^3/d x 11.28 yr x 365 d/yr x 10^{-3}). Neurobehavioral analysis revealed significant dose-response effects of arsenic exposure on CPT (P = 0.005), PM (P = 0.009) and SA (P = 0.0001), but not on SD (P = 0.23). A multiple linear regression analysis of the dose-response relationship between cumulative arsenic exposure and neurobehavioral endpoints showed a strong arsenic effects for CPT (low exposure group, P = 0.001), PM (high exposure group, P = 0.003) and SA (high and low exposures, P = 0.0001). This study is limited by low numbers but seems in line with other findings of As-induced CNS effects. The authors note that "the central nervous system of child and adolescents might be more vulnerable than adult to neurotoxicant". Although no dose-response relationship between As exposure and nerve conduction velocities was observed, the authors could not exclude the possibility of peripheral nerve dysfunction.

Wasserman *et al*. (2004) conducted a cross-sectional study of intellectual function in 201 As-exposed 10-year old children in Bangladesh. Children's intellectual function was assessed with tests drawn from the Wechsler Intelligence Scale for Children version III including Verbal, Performance, and Full-Scale raw scores. Children provided urine for arsenic and creatinine and blood samples for blood lead and hemoglobin measurements. After adjustment for sociodemographic covariates such as maternal education, height and head circumference, and waterborne levels of manganese (Mn), As in drinking water was associated with reduced intellectual function, in a dose-dependent manner. Children exposed to water arsenic of > 50 μg/L had significantly lower Performance and Full-Scale scores than did children with water As levels < 5.5 μg/L. Using the Full-Scale raw score, As water concentrations of 10 and 50 μg/L were associated with decrements of 3.8 and 6.4 points, respectively. The relationships between urinary arsenic concentration (μg As/g creatinine) and child intellectual function were not statistically significant but were in the expected (negative) direction (Full-Scale, P = 0.09; Performance, P = 0.14; Verbal, P = 0.11). Since there was no standard of intelligence for use in Bangladesh these decrements could not be directly equated with U.S. standard IQ points. However, "other simpler predictors of child intellectual function, such as maternal education and child height and head circumference, were significantly related to intellectual raw scores in the expected directions." In this study, as in others of this type exposure is inferred from water concentration.

Smith *et al*. (1998) studied lung and urinary bladder cancer mortality in a region of northern Chile (Region II, Antofagasta) where the residents were exposed to arsenic in their drinking water. Arsenic levels ranged from a population weighted average of 570 μg/L between 1955 and 1969 to 100 μg/L by 1980. Standardized mortality ratios (SMRs) were estimated for Region II as follows. Census data were used to calculate the person-years at risk during 1989-1993 by 10-year age groups, for men and women separately. National mortality data were obtained for 1991, the midpoint of the study period, and age- and sex-specific mortality rates were calculated for each cause of death of interest for the rest of Chile excluding Region II. The expected number of deaths was then calculated for Region II by multiplying the rest of the Chile 1991 age- and sex-specific mortality rates by the person-years at risk for residents in Region II for the period 1989-

1993. Standardized mortality ratios were estimated by dividing observed deaths by expected deaths. Statistical tests of significance were based on the Poisson distribution, and 95 percent confidence intervals were calculated using exact methods.

The SMRs (observed/expected deaths) for bladder, kidney, liver, and skin cancers, and all other cancers combined, were not related to age in either sex. However, lung cancer mortality ratios were particularly high in younger men aged 30-39 yr (SMR = 11.7, 95 percent C.I. 6.4-19.6, $P < 0.001$). The estimated SMRs were not as elevated in all groups. The values for the subsequent 10-year age groups were: 5.9; 4.9; 2.9; 4.0; 2.8; and 3.8 for the total with a 95%CI of 3.5-4.1. Also observed was a decreasing trend in chronic obstructive pulmonary disease deaths (COPD), with higher rates among younger men, particularly those aged 30-39. Four COPD deaths were reported among men (0.8 expected), and six deaths among women (0.1 expected). These ten individuals who died of COPD would have been young children at the time of peak arsenic water levels in 1955-1970. Smoking was accounted for but not in men and women separately.

In a later study Smith *et al*. (2006) reported increased mortality from lung cancer and bronchiectasis in young adults following arsenic exposures *in utero* and in early childhood. For subjects born just before the high exposure period (1950-1957) and exposed in early childhood the SMR for bronchiectasis was 12.4 (95% C.I., 3.3-31.7; $P < 0.001$). For those born during the high exposure period (1958-1970) with likely *in utero* and early childhood exposure the SMR for bronchiectasis was 46.2 (C.I., 21.1-87.7; $P < 0.001$). The authors conclude that "exposure to arsenic in drinking water during early childhood or *in utero* has pronounced pulmonary effects, greatly increasing subsequent mortality in young adults form both malignant and nonmalignant lung disease."

Additional evidence supporting a link between childhood arsenic exposure and subsequent lung disease comes from autopsies of children in the affected area. The results of five autopsies of children, who died in 1968 and 1969 in Antofagasta and showed skin lesions and other evidence of arsenic poisoning, also showed lung abnormalities in four of the children. Two of these cases exhibited interstitial fibrosis (Rosenberg, 1974). Also, a survey of 144 children in Antofagasta with skin pigmentation due to arsenic exposure reported a history of bronchopulmonary disease 2.5-fold more frequent than children with normal skin (15.9 vs. 6.2 percent, respectively) (Borgono *et al*., 1977).

Chronic exposure to arsenic has been associated with decreased birth weight and an increased rate of spontaneous abortion in female smelter workers. However, this association is confounded by the presence of other toxicants in the smelting process, including lead (Nordstrom *et al*., 1979). Anemia and leukopenia have been reported in infants ingesting approximately 3.5 mg As/day in contaminated milk over a period of 33 days (Hammamoto, 1955).

Premature birth and subsequent neonatal death was reported in a single individual following ingestion of arsenic (Lugo *et al*., 1969).

Ihrig *et al*. (1998) conducted a hospital-based case-control study of stillbirths and environmental arsenic exposure using an atmospheric dispersion model linked to a geographical information system. They collected data on 119 cases and 267 controls in a central Texas area including a facility with 60-year history of arsenic-based agricultural product manufacture. Four exposure

groups were categorized (0; < 10 ng/m^3; 10-100 ng/m^3; and > 100 ng/m^3). For the period 1983-93 they fit a conditional logistic regression model including maternal age, race/ethnicity, parity, income group, exposure as a categorical variable, and exposure-race/ethnicity interaction. Effects were only seen in the Hispanic group with the medium exposure group having a prevalence odds ratio and 95% confidence interval of 1.9 (0.5-6.6) and the high exposure group 8.4 (1.4-50.1). The authors postulate a possible influence of a genetic polymorphism affecting folate metabolism in Hispanic populations possibly leading to increased neural tube defects and stillbirths. Small numbers limits this study; for example, there were only seven cases in the high exposure group and five of these were Hispanic.

Von Ehrenstein *et al*. (2006) studied pregnancy outcomes, infant mortality, and arsenic exposure via drinking water in West Bengal, India. The reproductive histories of 202 women were reviewed including measurements of 409 drinking water wells. The total number of pregnancies was 660 and the number of live births plus stillbirths was 558. Odds ratios for spontaneous abortion, stillbirth, neonatal mortality (death in the first month) and infant mortality (death in the first year) were estimated by logistic regression. Exposure to arsenic concentrations ≥ 200 μg/L during pregnancy was associated with a six-fold increased risk of stillbirth after adjustment for potential confounders (OR = 6.07; 95% C.I. 1.24-24.0, p = 0.01). The odds ratio for neonatal death was 2.81 (95% C.I. 0.73-10.8). No significant associations were found for arsenic exposure and spontaneous abortion (OR = 1.01; 95% C.I. 0.38-2.70) or overall infant mortality (OR = 1.33; 95% C.I. 0.43-4.04). Arsenic related skin lesions were observed in12 women who had increased risk of stillbirth (OR = 13.1; 95% C.I. 3.17-54.0).

6.3 Subchronic and Chronic Toxicity to Experimental Animals

Female albino rats (20 per group) were exposed to 0, 1.3, 4.9, or 60.7 μg As_2O_3/m^3 as aerosol continuously for 3 months (Rozenshtein, 1970). Decreased whole blood sulfhydryl group content, histological changes in the brain, bronchi, and liver, changes in conditioned reflexes, and changes in chronaxy ratio were observed in both the high- and mid-dose groups. Among animals in the high dose group, eosinophilia, decreased blood cholinesterase activity, decreased serum sulfhydryl content, and increased blood pyruvic acid were observed. No significant changes were observed in the low-dose group.

Male mice (8-10 per group) were exposed to 0, 0.5, 2.0, or 10.0 ppm sodium arsenite in drinking water for 3 weeks followed by a 28-day recovery period (Blakley *et al*., 1980). The primary immune response of the spleen (as indicated by changes in IgM-production assayed by plaque-formation) was suppressed at all dose levels. The secondary immune response was also suppressed at all dose levels as indicated by a decrease in the number of IgG producing cells.

Male Sprague-Dawley rats (7-28 per group) were exposed to 0, 40, 85, or 125 ppm sodium arsenate in drinking water for 6 weeks (Brown *et al*., 1976). Rats from all arsenic exposed groups showed increased relative kidney weights, decreased renal mitochondrial respiration, and ultrastructural changes to the kidney.

Male ddY mice (number not stated) received 0, 3, or 10 mg As_2O_3/kg/day orally for 14 days and were examined for changes in concentrations of monoamine-related substances in various brain regions and for changes in locomotor activity (Itoh *et al*., 1990). Locomotor activity was

increased in the low-dose group and decreased in the high-dose group. Several monoamine-related compounds were altered in both dose groups in the cerebral cortex, hippocampus, hypothalamus, and corpus striatum. The study indicates an effect of arsenite on brain chemistry but is inconclusive with respect to dose response.

Male and female Wistar rats (7-10 per group) were treated from age 2 to 60 days by oral gavage with daily administration of 0 or 5 mg As/kg body weight (as sodium arsenate) (Nagaraja and Desiraju, 1993; 1994). After 160 days, body weights, brain weights, and food consumption were decreased in the arsenic exposed group. Acetylcholinesterase (AChE) and GAD activity and GABA levels were decreased in the hypothalamus, brain stem, and cerebellum during the exposure period; all but AChE activity returned to normal during the post-exposure period. Changes in operant conditioning were also observed among the exposed animals.

Female Holtzman rats (>5 per group) were treated with 0, 100, 500, 1000, 2000, or 5000 ppm As_2O_3 in feed for 15 days (Wagstaff, 1978). Hexibarbitone sleeping time was altered in all arsenic exposed groups. Body weight and feed consumption were decreased among animals in the groups exposed to ≥ 500 ppm As_2O_3. Clinical signs of toxicity observed among arsenic exposed animals included roughened hair, diarrhea, and decreased physical activity.

Male Sprague-Dawley rats and C57 black mice (12 per group) were treated with 0, 20, 40, or 85 ppm sodium arsenate in drinking water for up to 6 weeks (Woods and Fowler, 1978). Among arsenic exposed rats, heme synthetase activity was decreased in all exposed groups. Among animals exposed to ≥ 40 ppm sodium arsenate, hepatic ALA synthetase activity was decreased and urinary uroporphyrin and coproporphyrin were increased. Among exposed mice, heme synthetase activity was decreased and uroporphyrinogen I synthetase activity was increased in all exposed groups. Among animals exposed to ≥ 40 ppm sodium arsenate, urinary uroporphyrin and coproporphyrin were increased.

Administration of 3.7 mg As_2O_3/kg/day to Rhesus monkeys for 12 months did not result in any neurologic change detectable by an EEG (Heywood and Sortwell, 1979). Two of the 7 animals exposed to this concentration died before the conclusion of the 52-week period. Of the surviving animals, two were retained for a 52-week recovery period after which they were sacrificed and necropsied. No significant changes in organ weights or gross appearance were noted.

7. Toxicity of Arsine

7.1 Toxicity to Adult Humans

Numerous case reports of accidental arsine poisoning exist in the literature, but reliable estimates of concentrations during acute human intoxication do not exist. This is due in large part to the insidious nature of arsine toxicity - arsine is a colorless gas, has a mild odor at low concentrations, produces no mucous membrane irritation, and usually results in delayed symptoms of toxicity (Klimecki and Carter, 1995). In mammalian systems, arsine primarily targets the erythrocyte and causes hemolysis and methemoglobinemia with acute exposure (NRC, 1984). Jaundice, hemoglobinuria, anuria, hepatic and renal damage, anoxia, and anemia are secondary effects resulting from hemolysis. Before the advent of dialysis, there were no reports of patients surviving if renal failure developed (Buchanan, 1962). Other acute symptoms reported include

headache, weakness, dizziness, dyspnea, nausea, vomiting, diarrhea, and abdominal cramping (Klimecki and Carter, 1995). Central and peripheral nervous systems may be affected by acute arsine exposure, leading to agitation, disorientation, hallucinations, psychopathologic abnormalities, and peripheral nerve degeneration (Frank, 1976; Klimecki and Carter, 1995). The psychopathologic and peripheral abnormalities are thought to be secondary to the conversion of arsine to arsenate or arsenite. The first signs and symptoms of toxicity, hemoglobinuria and/or nausea, are usually delayed 2 to 24 hours following exposure (Kleinfeld, 1980).

A case report documents hemolytic anemia, hematuria, and renal failure following intermittent exposure to arsine gas over 2.5 hours (Parish *et al*., 1979). Symptoms of gastrointestinal distress, headache, and malaise were also reported following this exposure. The concentration of arsine gas sampled 3 days after exposure was 0.1 ppm (0.3 mg/m³), but the concentration at the time of poisoning was unknown. Another typical accidental poisoning resulted when 2 men were exposed to arsine gas in a metal smelting works (Coles *et al*., 1969). Symptoms included nausea, vomiting, red urine, generalized aching, shivering, epigastric pain, and jaundice. However, the more severely affected worker developed symptoms within 1 hour of exposure while the other did not develop symptoms for 24 hours. The more severely affected worker developed acute renal failure that required peritoneal dialysis.

In an occupational study, the highest average concentration of arsine recorded in a battery formation area of a battery manufacturing plant was 20.6 µg/m³ (0.006 ppm) (Landrigan *et al*., 1982). Elevated levels of urinary arsenic were observed in some workers but effects on the hematopoietic system were apparently not examined.

A study by Williams *et al*. (1981) collected personal and area air samples after 2 workers exhibited symptoms of arsine poisoning while restoring a large 19th century painting. Symptoms included headaches, nausea, weakness, vomiting, and red urine. The control-corrected air concentration of arsine ranged from 0.010 to 0.067 mg/m³. While these concentrations are below the OSHA PEL (permissible exposure level) 8-hour TWA (time weighted average) of 0.2 mg/m³, the results may indicate that these workers are sensitive responders or that humans in general may be more sensitive to the effects of arsine than experimental animals. However, the air samples may not represent the actual concentration of arsine that caused the symptoms of poisoning in the workers since the workplace air was not analyzed for arsine until after symptoms were reported. The study also notes that 'appreciable concentrations' of lead and arsenic were found in the workplace air.

No studies were identified addressing the chronic toxicity of arsine in humans.

7.2 Toxicity to Infants and Children

No studies were identified allowing quantitative assessment of arsine toxicity in infants and children. Arsine's mode of toxic action is not completely understood but appears to involve binding to erythrocyte sulfhydryl groups followed by intracellular ion loss and hemolysis (Rael *et al*., 2000). Clinical treatment of arsine poisoning usually involves exchange transfusion. It seems plausible that infants and children would be more sensitive to the irreversible hematotoxicity of arsine than adults due to their greater breathing rate per unit body weight.

7.3 Toxicity to Experimental Animals

A number of studies were reviewed to understand the time-concentration relationship of arsine lethality. The most complete and relevant study was the IRDC (1985), which allowed determination of 1% and 5% lethality benchmark doses for exposure durations of 0.5 to 4 hours in rats. The most important acute non-lethal effects noted were hemolysis and reticulocytosis (Peterson and Bhattacharyya, 1985). Longer term effects of arsine also involved significant changes in hematological parameters (hemoglobin and mean corpuscular volume) (Blair, 1990).

LC_{50} values (estimate of concentration resulting in 50 percent mortality of exposed animals) reported by Gates (1946) are as follows: 120-210 ppm (380-670 mg/m³) for 10 minutes in rats, 110 ppm (350 mg/m³) for 30 minutes in dogs (equivalent to 190 ppm (610 mg/m³) for 10 minutes), and 200-300 ppm (640-960 mg/m³) for 10 minutes in rabbits. An LC_{50} in mice was reported as 31 ppm (99 mg/m³) for a 50-minute exposure (Levvy, 1947). The survival time of the fatalities (4 days) was reported to be more or less independent of exposure concentration (2500 mg/m³ to 25 mg/m³) and exposure duration.

The study by Levvy (1947) in mice varied exposure durations for each given concentration of arsine. Because the mortality data were not presented in conventional form by the standard LC_{50} method, the data were normalized to a 1-hour exposure using the modified form of Haber's equation (as described in Section 5.7.1 of the TSD):

$$C^n \times T = K,$$

where C = concentration, T = time, K = a constant determined at a given C, T and the exponent n is a constant determined experimentally. The exponent "n" of 1.8 was determined by varying the term n in a log-normal probit analysis (Crump and Howe, 1983; Crump, 1984) until the lowest chi-square value was achieved. Fifty-four data points were used to estimate the exponent n because these points were of sufficient duration ($\geq$ 5 minutes) and resulted in the best chi-square fit for the line and obvious heterogeneity (Table 7.3.1). This relationship indicates that the toxicity of arsine varies approximately with the product of the square of concentration times time rather that simply concentration times time.

Table 7.3.1 Arsine Mortality in Mice: Results from Levvy (1947) and 1-Hour Adjusted Concentrations Using Haber's Equation (C^n x T = K, where n = 1.8).

Concentration (ppm)	Exposure Duration (min)	Mortality (no. died/total)	1-Hour Adjusted Concentration (ppm)
157*	10	30/30	58
	5	28/30	39
	2.5	17/30	27
	1.7	0/30	22
78.4*	15	21/30	36
	9	10/30	27
31.4	70	30/30	34
	50	15/30	28

** Shaded rows include data used for determination of the ED_{05} and BD_{05}*

Craig and Frye (1988) reported a 4-hour LC_{50} of 42.6 ppm in rats. However, when the rats were separated by sex for statistical purposes, there was slightly greater mortality among females than males (38.9 ppm LC_{50} for females vs. 46.8 ppm LC_{50} for males). No abnormalities were seen at necropsy except red discharge from nose, mouth, and genitalia at the higher concentrations. A concentration-related suppression of body weight gain was observed during the first week of the 14-day post-observation period.

The most comprehensive arsine lethality study was undertaken by IRDC (1985). LC_{50}s of 240, 178, and 45 ppm were determined in rats (10 rats/sex/group) for 30 minute, 1 hour, and 4-hour exposures, respectively. Deaths generally occurred within 3 days following 30-minute exposure to arsine. As in the previous study (Craig and Frye, 1988), there was slightly greater mortality in females than males. Adverse effects noted during exposure included dyspnea, while effects noted post-exposure included a concentration-related increase in hematuria, dark material around the head or the anogenital area, and pallor of ears, eyes, and feet. The higher concentrations resulted in weight loss immediately following exposure, suppressed weight gain during the first week and compensatory weight gains during the second week post-exposure. Necropsy on animals that died showed red, yellow or orange fluid in the bladder, stomach, or intestine, and discoloration of the kidneys, lungs, and liver.

Data in the IRDC (1985) report were used to determine the exponent "n" in the equation C^n x T = K. This was done by varying the term n in a log-normal probit analysis (Crump, 1984; Crump and Howe, 1983) until the lowest chi-square value was achieved. The value of "n" for extrapolation to 1-hour exposure was dependent on exposure duration. For extrapolation from 30 minutes to 1-hour exposure, n = 2.2; for extrapolation from 4-hours to 1-hour exposure, n = 1.0.

Table 7.3.2 contains the studies which provided adequate raw mortality data from which a maximum likelihood estimate corresponding to 5% lethality (ED_{05}) and benchmark dose at the 95% lower confidence interval of the ED_{05} and ED_{01} (BD_{05} and BD_{01}, respectively) could be determined.

Table 7.3.2 Animal Lethality Benchmark Dose Determinations in ppm for Arsine

Reference	Species	Exposure Time (min)	LC_{50} 60 min[1]	ED_{05} 60 min[1]	BD_{05} 60 min[1]	BD_{01} 60 min[1]
IRDC, 1985	rat	30	175	120	105	86
	rat	60	178	112	88	66
	rat	240	181	118	101	80
Craig and Frye, 1988	rat	240	170	125	102	84
Levvy, 1947	mice	varied[2]	29	20	16	13

[1] Exposure time was extrapolated to 60 minutes, if needed, using a modification of Haber's equation ($C^n * T = K$). For rats, n = 2.2 for extrapolation from 30 minutes to 1-hour, or n = 1.0 for extrapolation from 4 hours to 1-hour; for mice, n = 1.8.

[2] Lethality data for 5 exposure durations were pooled and normalized to a 1-hour exposure using the equation $C^n \times T = K$ (see Table 1).

In other experimental animal studies, a reduction in hematocrit as a function of arsine concentration was observed in mice following a 1-hour exposure (Peterson and Bhattacharyya, 1985). A LOAEL of 9 ppm (29 mg/m³) and a NOAEL of 5 ppm (16 mg/m³) were reported. The demarcation between the NOAEL and LOAEL for this non-lethal effect was well defined, not only among the exposure groups (5 ppm vs. 9 ppm), but also among individual mice in each exposure group (Peterson, 1990). Hematologic recovery of the surviving mice was gradual but nearly complete within 11 days after exposure (Peterson and Bhattacharyya, 1985). The study also reported a NOAEL of 15 ppm (100% survival) and LOAEL of 26 ppm (100% lethality) for lethality.

A continuous benchmark dose analysis of these data was performed. The full data set on hematocrit reduction 24 hours after exposure gave a BMD_{05} of 7.81 ppm and a $BMDL_{05}$ of 5.2 ppm (quadratic continuous model fit P= 0.16). The only other data sets that were adequately fit were the 24 hour increase in reticulocyte count (%) with the 11 and 26 ppm outliers removed (power continuous model, P = 0.50) and the 5 days values with the 9 ppm outlier removed (cubic continuous model, AIC = 61.8). Several response levels were evaluated including 25% relative, 1 and 2 % absolute increases and 1 and 2 standard deviations. The latter SD levels were closest to the minimal significant increase levels and exceeded the control plus one control SD values of 0.88 ppm (24 hr) and 2.0 (5 days). For a 1 SD response level at 24 hours the BMD_{1SD} = 3.29 ppm and the $BMDL_{1SD}$ = 2.17 ppm. The values for 2SD were BMD_{2SD} = 4.69 ppm and $BMDL_{2SD}$ = 3.50 ppm. For the 5 days data set the BMD_{2SD} = 4.32 ppm and the $BMDL_{2SD}$ = 2.70 ppm. Reticulocytosis may be a more sensitive indicator of adverse hematologic effects of arsine exposure than hematocrit reduction.

A subchronic study in male and female rats and female mice (Fowler *et al*., 1989) supports the sharp increase in dose-response noted by Peterson and Bhattacharyya (1985). All treatment groups exposed to arsine (6 hr/day, 5 days/week) at concentrations of 10 ppm and above showed 100 percent mortality within 4 days while those exposed to 5 ppm or less showed no mortality or overt signs of toxicity. Other effects observed included a dose-related increase in spleen weight and a slight increase in liver weight. Blood samples taken at necropsy showed a slight dose-

related decrease in hematocrit and a marked dose-related increase in the activity of red blood cell ALAD (δ-aminolevulinic acid dehydratase).

In a 90-day study, male and female mice were exposed to 0, 0.025, 0.5, and 2.5 ppm arsine gas for 6 hours/day, 5 days/week (Blair *et al*., 1990). After 5, 15, and 90 days, blood was collected for hematologic analysis. Exposure to 2.5 ppm had significant effects on all hematological parameters for nearly the entire exposure period, while 0.5 ppm caused only a few significant changes in hematological parameters at day 90 of exposure (decreased hemoglobin in males and increased MCV in females). Exposure to 0.025 ppm was without effect.

A continuous benchmark dose analysis was performed on the data sets of Blair *et al*. 1990. Adequate fits to the hematocrit data were obtained with the linear and quadratic models with $BMDL_{025}$ (relative risk) values ranging from 0.128 to 0.894 ppm (P values for model fits of 0.11 to 0.96). Absolute reticulocyte count increases gave continuous $BMDL_{10}$'s ranging from 0.22 to 0.68 ppm with linear and quadratic models (P values of 0.31 to 0.99). However, due to the poor dose spacing, essentially a missing dose level between 0.025 and 0.5 ppm, these results are considered inconclusive in determining an alternative NOAEL to 0.025 ppm.

7.4 Developmental and Reproductive Toxicity

In an unpublished study, workers in one semiconductor plant were reported to have a 39% rate of miscarriage, almost twice the national average (Sanger, 1987). Workers were exposed to unidentified levels of arsine gas, but other possible exposures were not identified.

A developmental toxicity study exposed pregnant rats and mice to 0.025, 0.5, or 2.5 ppm (0.079, 1.5, or 7.9 mg/m³) arsine for 6 hours per day on gestation days 6 through 15 (Morrissey *et al*., 1990). The rats exposed to 2.5 ppm exhibited a significant increase in fetal body weight, but no other endpoints of developmental toxicity were observed. The incidence of malformations observed in arsine exposed mice at 0.025 ppm (exencephaly) and at 2.5 ppm (unfused eyelids) was not significantly different from control mice.

8. Derivation of Reference Exposure Levels

8.1 Acute Reference Exposure Level for Inorganic Arsenic

Study	Nagymajtenyi *et al*., 1985
Study population	pregnant mice
Exposure method	maternal inhalation exposure
Exposure continuity	
Exposure duration	4 hours per day on gestation days 9, 10, 11, and 12
Critical effects	decreased fetal weight
LOAEL	0.26 mg/m³ As_2O_3 (0.197 mg As/m³)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	n/a
Human Equivalent Concentration	n/a
LOAEL uncertainty factor (UF_L)	10 (no NOAEL)
Subchronic uncertainty factor (UFs)	n/a
Interspecies Uncertainty Factor	
Toxicokinetic (UF_{A-k})	√10 (animal study)
Toxicodynamic (UF_{A-d})	√10 (animal study)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF_{H-k})	√10 (remaining interindividual variation: study considered effects on fetus or infant)
Toxicodynamic (UF_{H-d})	√10 (interindividual variation)
Cumulative uncertainty factor	1,000
Reference Exposure Level	**0.0002 mg As/m³ (0.20 μg As/m³,)**

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 in the Technical Support Document). The most appropriate study for the basis of an acute REL for arsenic is Nagymajtenyi *et al*. (1985). This study was selected since it measured a sensitive toxicological endpoint with a relevant route of exposure, and the experimental design and reporting were considered adequate (as specified in the Non-cancer Risk Assessment technical support document, Section 4.1.1). It involved a significant number of animals exposed by inhalation to three dose levels plus a control. Unfortunately, no NOAEL was obtained. However, a significant dose-related reduction in fetal weight and increased incidences of intrauterine growth retardation, skeletal malformations, and hepatocellular chromosomal aberrations were observed in mice following maternal inhalation exposure to 200 μg As/m^3 (260 μg As_2O_3/m^3) for 4 hours on gestation days 9, 10, 11, and 12 ($p<0.05$). The most sensitive effect, decreased fetal weight, was observed at 200 μg As/m^3, so 200 μg As/m^3 was taken as a LOAEL. Maternal toxicity data were not reported. This study is used as the basis of the acute REL:

0.2 $mg/m^3/1000$ = 0.0002 mg/m^3 = 0.2 μg As/m^3 (equivalent to 0.065 ppb arsine gas)

No temporal adjustment was made for the critical study since the critical period of exposure for a developmental effect may be very short relative to the study duration (OEHHA, 2007). The study concentration with appropriate uncertainty factors is a "not to exceed" value. An uncertainty factor of 10 (UF_L) was used to account for the lack of a no observed adverse effect level (NOAEL). A second uncertainty factor of 10 was used to account for interspecies differences between the test species and humans. This factor is the product of two components addressing pharmacokinetic (UF_{A-k}) and pharmacodynamic (UF_{A-d}) differences, each assumed to be the $\sqrt{10}$. A final uncertainty factor of 10 was applied to address human interindividual differences in pharmacokinetics (UF_{H-k}) and pharmacodynamics (UF_{H-d}) also assumed to be $\sqrt{10}$ each. The overall uncertainty of extrapolating from 4-hour exposures in mice (LOAEL) to no anticipated effects in humans is 1000 as noted in table above and the calculation of the acute REL. The rationale for the choice and value of uncertainty factors used by OEHHA is provided in the Non-cancer Risk Assessment technical support document (Section 4.4.3).

Inorganic arsenic (oxides) are listed as developmental toxicants under the California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). The studies reviewed in this document support the conclusion that exposure to inorganic arsenic may affect fetal weight, spontaneous abortion, neonatal death and postnatal neurological development.

In humans, the logarithm of infant mortality (death) increases linearly as birth weight decreases from 3500 to 1000 grams (Hogue *et al*., 1987; Rees and Hattis, 1994). This log-linear relationship exists on both sides of the weight (2500 g) conventionally used as a cutoff defining low birth weight. There is no evidence for a threshold. Thus any reduction in fetal weight is a cause for concern since it increases mortality. In the absence of certainty, OEHHA takes the health protective approach that the reduced weight effect in the animal fetuses may be biologically significant, particularly when viewed from a population perspective.

8.2 Inorganic Arsenic 8-Hour Reference Exposure Level

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures which might include daily occupational, in-home or in-school exposures. (see Section 6 in the Technical Support Document).

Due to the possibility of repeated exposure and the relatively slow clearance of arsenic compounds, the 8-hour REL is taken to be equivalent to the chronic REL. The half-life of the initial exponential phase of excretion of arsenic after a single dose is typically between one and two days, but there are also several much slower excretion processes. So a single exposure to arsenic would take several days to be cleared, mainly via urinary metabolites. Repeated exposures can significantly prolong the clearance of arsenic as the internal dose accumulates, so that in terms of internal dosimetry it would be difficult to distinguish repeated periodic exposure from chronic exposure scenarios. An individual exposed daily via air and/or drinking water might show very similar urinary arsenic excretion to another individual exposed only periodically at work, school etc.

8.3 Inorganic Arsenic Chronic Reference Exposure Level

Study	Wasserman *et al*. (2004); Tsai *et al*. (2003)
Study population	201 children 10 years of age
Exposure method	drinking water
Exposure continuity	continuous
Exposure duration	9.5 to 10.5 years
Critical effects	Decrease in intellectual function, adverse effects on neurobehavioral development
LOAEL	0.23 μg As/m^3 based on est. LOAEL of 2.27 μg/L (Wasserman *et al*., 2004; see Section 8.3.1.1)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	none, exposure considered continuous
Human equivalent concentration	n/a
LOAEL uncertainty factor (UF_L)	3 (LOAEL estimated by quantitative analysis of study data)
Subchronic uncertainty factor (UFs)	1 (default: duration >8% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (remaining interindividual variation: study considered effects on 10 year-old but not infant)
Toxicodynamic (UF_{H-d})	√10 (default, interindividual variation)
Cumulative uncertainty factor	30
Inhalation Reference Exposure Level	**0.015 μg As/m^3**
Oral Reference Exposure Level	0.0035 μg/kg-d

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

8.3.1.1 Child Based Values

A number of studies have indicated potentially greater toxicity of arsenic exposure during childhood (see below). Although some PBPK modeling has been applied to inorganic arsenic and its methyl metabolites, the modes of toxic action and relevant internal dosimetry are not sufficiently understood at present to use this modeling directly in REL development. In this section we compare quantitative analyses of dose-responses and LOAELs in key studies involving arsenic exposures in children. Health protective exposure levels derived from these analyses will be compared with similar analyses from studies in adults in the following section.

The study of Wasserman *et al*. (2004) indicated a dose-response of decreasing Full-Scale intellectual function raw scores with increasing drinking water arsenic exposure in 10-year olds. The values in their Fig.2 give an exact fit to a quadratic model ($Y = Y_0 + aX + bX^2$; Y_0 intercept = 0, a = -0.443, b = 0.0063, $R^2 = 1.0$) with a low dose slope of –0.44 points/μg/L. Assuming an adverse effect level of one point loss, then the corresponding arsenic concentration can be calculated as:

-1point/-0.44 point/μg/L = 2.27 μg/L.

This level might be equivalent to a LOAEL. Further, assuming water intake of 1 Liter/day (L/d) and essentially complete intestinal absorption, this can be converted to an intake of 2.3 μg/d. If we assume a drinking water intake based on the 95% upper confidence level (UCL) for U.S. children aged 1 to 10 years of 1564 mL/day the intake would be somewhat higher at 3.6 μg/d (OEHHA, 2000; Table 8.3). Since 10-year old males would inhale about 9.9 m^3/d (OEHHA, 2000), if airborne arsenic were 100% absorbed, this oral effect level would be equivalent to an inhalation level of 2.3 μg/day/9.9 m^3/day = 0.23 μg/m^3. Assuming a more realistic inhalation absorption of 50 % would give a value of 0.46 μg/m^3. Applying a 3-fold UF for an estimated LOAEL based on a quantitative dose response analysis (a higher value would be used without a dose response analysis) and 10-fold for inter-individual variation since only 10-year olds were studied, a health protective air concentration of 0.015 μg/m^3 can be calculated. An oral value based on the average study body weight of 21.9 kg and 100% oral absorption would be 2.3 μg/d/21.9 kg = 0.105 μg/kg-day. Applying the same overall uncertainty factor of 30 the oral health protective value would be 0.105 μg/kg-day/30 = 0.0035 μg/kg-day.

The data of Tsai *et al*. (2003) for 13 year old children gave dose response relationships for arsenic exposure metrics of ppb As in drinking water and cumulative arsenic intake (mg) vs. the pattern memory (PM) and switching attention (SA) endpoints (ms). A continuous benchmark response analysis for ppb As vs. ms test duration was conducted for PM (BMD_{05} = 49.75; $BMDL_{05}$ = 31.2 ppb) and SA (BMD_{05} = 28.81; $BMDL_{05}$ = 19.73 ppb) both using a linear model. For cumulative As intake the PM endpoint data were similarly fit by a linear model (BMD_{05} = 194.1; $BMDL_{05}$ = 122.7 mg) and the SA data by a polynomial (quadratic) model (BMD_{05} = 39.1; $BMDL_{05}$ = 25.4 mg; see Fig. 1). The SA endpoint appears to be the most sensitive. Based on the SA $BMDL_{05}$ of 19.7 ppb and 1 L/d drinking water intake a minimum effect level of 19.7 μg/d is estimated. If we assume a drinking water intake, based on the 95% UCL for U.S. children aged 11 to 19 years of 2.143 L/d the intake would be 2-fold higher at 42.2 μg/day (OEHHA, 2000; Table 8.3). Using uncertainty factors of 10 for interindividual variation and 3 for extrapolation from a minimum to a no effect level, a health protective intake of 19.7 μg/d/30 = 0.658 μg/d is calculated. Assuming inhalation of 10 m^3/d and 50 % absorption (default) this value can be converted to an inhalation value of 0.658 μg/day/(0.50 x 10 m^3/day) = 1.32 μg/m^3. Using the SA cumulative $BMDL_{05}$ of 25.4 mg As and 10 years exposure, an effect level of 25.4 mg/(10 yr x 365days/yr) = 6.96 μg/day is calculated. Using the same assumptions and UFs as above, an inhalation value of 0.044 μg/m^3 can be derived based on As concentration. The cumulative dose metric is a more accurate estimate of arsenic exposure than As water concentration, so the value of 0.046 μg/m^3 or 0.05 μg/m^3 (rounded) is preferred over the concentration based value. An oral value based on an average body weight for a 13-14 year old child (OEHHA, 2000) of 50 kg is 6.96 μg/day/50 kg = 0.139 μg/kg-d. Applying the same overall uncertainty factor of 30 would give 0.139 μg/kg-day/30 = 0.0046 μg/kg-day.

The quantal responses for skin lesions in young children (≤ 9 yr) and adolescents (10-19 yr) from Mazumder *et al*. (1998) were subjected to benchmark dose analysis. For young children, the quantal linear model adequately fit the data (X^2 = 6.1, P = 0.30) with a BMD_{01} = 54.4 μg/L and a $BMDL_{01}$ = 39.3 μg/L. For adolescents, the best fitting model was the log probit (X^2 = 0.77, P = 0.68) with a BMD_{01} = 77.3 μg/L and a $BMDL_{01}$ = 47.4 μg/L. These values are similar to the

analysis of all age groups combined (above) and application of a 10-fold UF for intraspecies variation seems adequate for these data. Thus the health protective intake for children for skin effects would be in the range of 3.9 to 4.7 μg/d for one liter/day water intake. For conversion to inhalation equivalent, young children are assumed to inhale 9.9 m^3/day and drink 1 liter/day and adolescents to inhale 14 m^3/day and drink 1.5 liter/day (OEHHA, 2000). It is further assumed that 50 percent of inhaled arsenic is absorbed via the pulmonary and gastro-intestinal routes. The resulting health protective values would be 0.68 to 0.79 μg/m^3.

A study in Thailand (Siripitayakunkit *et al*., 1999) related drinking water arsenic exposure, indicated by hair arsenic, to IQ in 529 six to nine year old children. A continuous benchmark dose response analysis of this data set gave a BMD_{05} = 0.035 μg As/g hair and $BMDL_{05}$ = 0.0155 μg As/g (polynomial model). A slope of –3.2 IQ points/μg/g was derived from the $BMDL_{05}$. Using the conversion factor of 0.01μg As/g hair/μg As/Liter of water (Kurttio *et al*. 1998), a decrease of 1 IQ point would be equivalent to chronic consumption of 30 μg As/L water (OEHHA, 2004). At one liter/day water consumption the 30 μg/d value is over an order of magnitude higher than the analogous estimate indicated by the Wasserman *et al*. (2004) study above. An inhalation value was derived as above: 30 μg/day/(10 m^3/day x 0.50 x 30UF) = 0.20 μg/m^3.

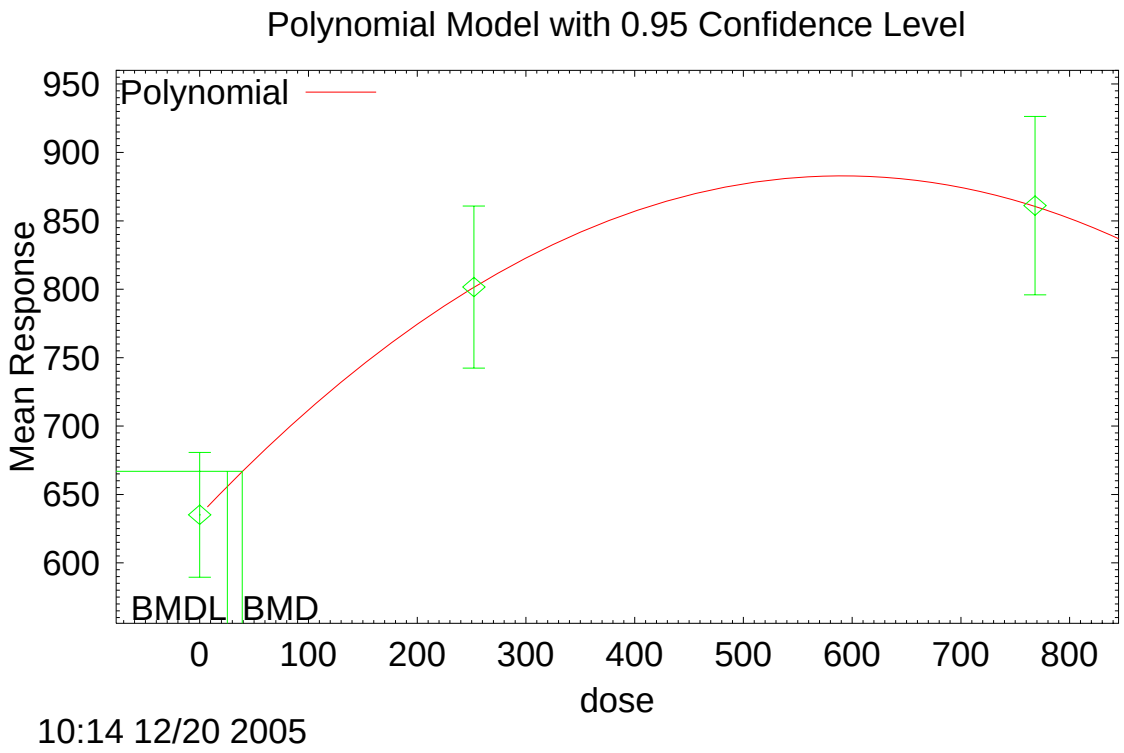
The visual perception data from Siripitayakunkit *et al*. (2001) was subjected to continuous benchmark dose analysis. The $BMDL_{035}$ of 2.40 μg/g hair (polynomial model) was near the low level mean minus one SD score (20.5), presumably an adverse effect level on visual perception as defined by the authors. The linear model gave a higher value (3.69 μg/g) but did not fit the data as well in the low exposure range. Using the conversion factor above, one liter per day water consumption, and a 30-fold cumulative UF results in a presumptive health protective intake of 8 μg/d for this endpoint (2.40 μg/g ÷ 0.01 μg/g/μg/Liter x 1 Liter/day ÷ 30UF = 8.00 μg/d). An inhalation value was derived as above: 8.0 μg/day/(10 m^3/day x 0.50) = 1.6 μg/m^3.

Chronic arsenic exposure appears to have adverse effects on intellectual development and visual perception in children. While the quantitation of these effects and the toxicological significance of the criteria selected are somewhat uncertain, OEHHA thinks they are sufficient to support a chronic reference exposure level (cREL). It is uncertain whether neurological effects are the most sensitive caused by chronic arsenic exposure in children. Additional studies in exposed children are needed to adequately quantify adverse effects. The values above are summarized in Table 8.3.1. The child-based values range from 0.015 to1.6 μg/m^3. The geometric mean of the three cognitive endpoint values (0.015, 0.20, 0.05) is 0.053 μg/m^3.

Table 8.3.1. Inhalation Values Derived from Human Child Studies

Study	Toxic Endpoint	Criterion	Value	Derived cREL, μg/m^3
Wasserman *et al*. (2004)	Intellectual function	One point loss	2.27 μg/d	0.015
Siripitayakunkit *et al*. (1999)	IQ	One point loss	-3.2 IQ/μg/g hair As	0.20
Siripitayakunkit *et al*. (2001)	Visual perception loss	LOAEL	240 μg/d	1.6
Mazumder *et al*. (1998)	Skin Lesions	LED_{01}	39-47 μg/d	0.68-0.79
Tsai *et al*. (2003)	Neurobehavioral effects	LED_{05}	7 μg/d	0.05
Smith *et al*. (2006)	Bronchiectasis mortality	LED_{01}	213 μg/d	1.4

Figure 8.3.1 Switching attention (ms) in 13-year old children versus cumulative arsenic intake in mg (Tsai et al., 2003).



Inorganic arsenic is apparently more potent in its neurotoxic effects in humans than in experimental animals. The values of 2.27 μg/day in Wasserman *et al* (2004) and 7 μg/day in Tsai *et al* (2003) for cognitive effects in 10-13 year-old children are much lower than brain

effects seen in animals e.g., 5 mg/kg-day in rats (Nagaraja and Desiraju, 1993; 1994) and 3.7 mg/kg-day in Rhesus monkeys (Heywood and Sortwell, 1979).

The bronchiectasis data from Smith et al. (2006) were subjected to benchmark dose analysis. A control value based on a background incidence rate of 0.04% (1/2500) and exposure of 40 µg As/L x 10 yr were used together with observed incidence values of 4/651 (90 µg As/L x 10 yr) and 9/488 (870 µg As/L x 13 yr). No statistically significant model fits were obtained. The best fitting model was the log probit ($X^2 = 4.95$, $P = 0.026$) which gave an LED_{01} ($BMDL_{01}$, 1% response) of 2.77 (mg/L) x yr. This value can be converted to an inhalation value of 1.42 µg/m^3 (2.77mg yr/L x 1000 µg/mg/(13 yr x 10 m^3/d x 30UF x 0.5) = 1.42 µg/m^3). This value has been added to Table 8.3.1 for comparison only due to the poor model fit.

8.3.1.2 Adult Based Values

In this section we review toxicological criteria from studies in adults that may serve as the basis for a chronic REL for inorganic arsenic or otherwise provide supporting information.

Studies in experimental animals show that inhalation exposure to arsenic compounds can produce immunological suppression, developmental defects, and histological or biochemical effects on the nervous system and lung, thus providing supportive evidence of the types of toxicity observed in humans. Among the inhalation studies, the lowest adverse effect level (LOAEL) was quite consistent:

245 µg As/m^3 for decreased bactericidal activity in mice (Aranyi *et al*., 1985);
200 µg As/m^3 for decreased fetal weight in mice (Nagymajtenyi *et al*., 1985); and
270 µg As/m^3 for decreased sperm motility in rats (Kamil'dzhanov, 1982).

Reports of human inhalation exposure to arsenic compounds, primarily epidemiological studies of smelter workers, indicate that adverse health effects occur as a result of chronic exposure. Among the targets of arsenic toxicity are the respiratory system (Lundgren, 1954), the circulatory system (Lagerkvist *et al*., 1986), the skin (Perry *et al*., 1948), the nervous system (Blom *et al*., 1985), and the reproductive system (Nordstrom *et al*., 1979). Occupational exposure levels associated with these effects ranged from 50 to 7000 µg As/m^3. These epidemiological studies suffer, however, from confounding as a result of potential exposure to other compounds, which limits their usefulness in the development of the chronic REL.

A single study showed effects occurring at 4.9 µg As_2O_3/m^3 (Rozenshtein, 1970). However, lack of detail with respect to endpoints and experimental design limits this study's usefulness for developing a Reference Exposure Level.

The cerebrovascular disease (CVD) and cerebrovascular infarct (CI) data of Chiou *et al*. (1997b) were subjected to benchmark dose analysis (BMD). The data were best fit using the quantal linear regression (QL) dose-response equation. Since the responses were of the order of 0.1 to 2 percent, the values calculated were for the 1 percent response (ED_{01}) and its 95% lower confidence limit (LED_{01}), rather than the usual 5 percent response values for the analysis of animal study data.

The values for CI were marginally better fit by the dose-response equation than those for CVD. Also the QL models gave better fits to the unadjusted data sets for both endpoints. The unadjusted ED_{01} and LED_{01} values with goodness of fit P value meeting the acceptable fit criterion of $P \geq 0.1$ were 359 and 189 μg/L for CVD and 268 and 166 μg/L for CI, respectively. Using the cumulative dose metric these values were 5.1, 3.0, 5.9, and 3.5 (mg/L)-yr, respectively. Due to the severity of these and other endpoints analyzed below, the uncertainty in the dose assignments (range mid-points instead of averages), and the fact that the chosen points of departure or LEDs were generally two-fold or more above concurrent control levels, the LED_{01} should be considered equivalent to a LOAEL for the purposes of risk assessment. Due to the severity of the CI endpoint, a 100 UF was used to derive a health protective water concentration of 0.1 to 0.3 μg/L based on the two dose metrics. For CVD with a 30 UF the corresponding values were 0.28 to 1.3 μg/L (for details of analysis see OEHHA, 2004). Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption, the corresponding inhalation values for these vascular effects would be for CI 0.10 to 0.33 μg/m^3 and for CVD 0.28 to 1.26 μg/m^3.

BMD analysis of the ISHD data from Chen *et al*. (1996) showed that these data were well fit by the QL dose-response equation (ED_{01} = 8.27 (mg/L)-yr, X^2 = 0.26, P = 0.88). The LED_{01} of 5.53 (mg/L)-yr should be considered an effect level for this endpoint. In this analysis the cumulative arsenic dose metric of (mg/L)-yr and resultant benchmark doses were divided by 70 yr to yield comparable lifetime drinking water concentrations of arsenic. Using a cumulative uncertainty factor of 100, a health protective concentration of 0.16 μg/L can be derived (OEHHA, 2004). Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption the corresponding health protective inhalation value for ISHD would be 0.16 μg/m^3.

The Chen *et al*. (1995) data on the association of hypertension (HT) and cumulative arsenic intake via drinking water were subjected to BMD analysis. The QL dose-response equation fit the unadjusted data well but was somewhat less than adequate for the adjusted prevalence values. The acceptable criterion for the X^2 goodness of fit test for the benchmark dose is $P \geq 0.10$. In the case of arsenic induced hypertension, the 10 percent effect level was chosen due to the higher background and greater dose response range compared to other human studies evaluated where 1% or 5% response levels were used. For HT the LED_{10} is considered an appropriate LOAEL for risk assessment. In the case of the adjusted data set, removal of the highest cumulative dose allows an acceptable fit of the QL equation with an LED_{10} of 7.4 (mg/L)-yr. The data of Rahman *et al*. (1999) were also analyzed. Both crude and adjusted data sets were well fit by the QL model with P values much greater than 0.1. The unadjusted LED_{10} value of 6.3 (mg/L)-yr from Bangladesh is quite similar to comparable value of 7.2 (mg/L)-yr from the Taiwan study (OEHHA, 2004). Health protective drinking water concentrations with a cumulative uncertainty factor of 30 ranged from 0.55 to 0.68 μg/Liter. Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption the corresponding health protective inhalation value for HT would be 0.55 to 0.70 μg/m^3.

The data of Chen *et al*. (2006) indicate a supralinear dose-response. The data were analyzed for benchmark response using metrics of time weighted average (TWA) and cumulative arsenic exposure of TWA times years of exposure or (mg/L)-yr. Systolic hypertension quantal responses of the first four quintiles of the overall population (N = 8726) were fit by the log-logistic model

of BMDS (v 1.4.1). The $BMDL_1$ values (1% response) of 71.5 µg/L and 0.66 (mg/L)-yr were obtained ($X^2 = 3.8$, P = 0.15, d.f. = 2). The pulse hypertension data were similarly fit using the longer-term exposure subpopulation (N = 6319). In this case the 10% response level was used for $BMDL_{10}$'s of 0.49 µg/L and 0.004 (mg/L)-yr ($X^2 = 4.45$, P = 0.11, d.f = 2). TWA BMDLs for systolic and pulse hypertension in arsenic exposed subpopulations with lower intakes of B vitamins were also evaluated. The $BMDL_{10}$ values for populations with low dietary folate ranged from 62 to 405 µg/L TWA. The results indicate a higher sensitivity of the pulse hypertension effect to low level arsenic than the systolic hypertension effect. The supralinearity of dose-response makes comparison with earlier studies problematic. For example, projected 10^{-4} extra risk levels for pulse and systolic hypertension from this study are at least an order of magnitude less than values seen earlier with Chen *et al*. (1995) or Rahman *et al*. (1999) although cumulative arsenic exposures were 5-10 times higher in the latter studies (Table 6). A cREL estimated from the 0.49 µg/L value above would be 0.0033 µg/m^3 (0.49 µg/L x 2L/d/(20m^3/d x 0.5 absorption x 30UF).

Similarly, the diabetes mellitus (DM) data of Lai *et al*. (1994) and Rahman *et al*.(1998) were analyzed. In this case, the QL dose-response model adequately fit both unadjusted and multivariate-adjusted prevalences. EDs and LEDs were determined for the 1 and 5 percent response levels. The LED_{05} for the adjusted values appear the best choice for a chronic criterion for arsenic-induced diabetes mellitus, i.e., 8.8 (mg/L)-yr from Lai *et al*. and 0.21 mg/L from Rahman *et al*. The health protective drinking water derived from these values with a cumulative UF of 30 were 0.84 and 1.4 µg/L, respectively (OEHHA, 2004). Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption, the corresponding health protective inhalation values for diabetes mellitus would be 0.85 to 1.4 µg/m^3.

In addition to the values noted above, an estimated LOAEL of 20 (mg/L)-yr for peripheral vascular disease from Tseng *et al*. (1996) was also included in this analysis. Using a cumulative UF of 30, a drinking water value of 1.9 µg/L was derived (OEHHA, 2004). Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption, the corresponding health protective inhalation value for peripheral vascular disease would be 1.9 µg/m^3. The study of Wang *et al*. (2002) on arsenic induced carotid atherosclerosis (subclinical) also gave an estimated LOAEL of 20 (mg/L)-yr and would yield the same health protective values.

The arsenic-induced skin keratosis and hyperpigmentation data of Mazumder *et al*. (1998) were analyzed as above (OEHHA, 2004). For both male and female skin keratosis data sets, adequate fits were obtained by the QL model with lower bound values (LED_{01}) of 49.6 µg/L for males and 124 µg/L for females. Adequate fits could not be obtained for both hyperpigmentation data sets with the models available in the benchmark dose program; however, the dose-response graphs appeared to be linear in the lower exposure groups with respective LED_{01}s of 18.9 and 34.7 µg/L. It appears that a single dose level outlier (125 µg/L) was largely responsible for the failure of the statistical test. Mazumder also included an assessment of skin keratosis and hyperpigmentation prevalence by dose per body weight. Using the dose metric of µg/kg-day, the skin hyperpigmentation data were still unable to be fit by the BMDS models. Therefore only the skin keratosis endpoint appears suitable for the development of a health protective value for

arsenic-induced noncancer effects. Using a cumulative UF of 30, a drinking water value of 1.7 μg/L was derived. Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption, the corresponding health protective inhalation value for skin keratosis would be 0.34 $\mu g/m^3$.

The skin lesion data of Rahman *et al*. (2006) was analyzed for benchmark response. The unadjusted data reported in Rahman's Table 3 was used with the mid points of the exposure concentration ranges (e.g., 5, 30, 100, 224, 450 μg/L) and the mean As exposures in Rahman's Table 4 (e.g., 9.8, 59.3, 127, 199, 344 μg/L). For the unadjusted male data, no adequate fit could be obtained. The female data was adequately fit by the quantal linear (P = 0.43) and log-logistic (P = 0.51) models. The latter giving a $BMDL_{10}$ of 6.28 μg/L with mid-point based exposure estimates, and the former giving a $BMDL_{10}$ of 108.2 μg/L with mean As concentrations. Similarly, for the cumulative As dose metric of (mg/L)-yr no adequate fit was obtained with the male data, while the female data were best fit by the log-probit model (P = 0.86) for a $BMDL_{10}$ of 2.80 (mg/L)-yr. Using the age and asset adjusted data with the average As concentrations, an adequate fit to the male data could be obtained with the multistage model if the top dose group was removed, $BMDL_{10}$ = 96.0 μg/L (X^2 = 0.60, P = 0.74). The female adjusted data set gave a lower BMDL of 65.4 μg/L despite the authors' finding that the males were more sensitive. This may simply reflect the difficulty of fitting the male data. In almost all cases, the BMDL values are lower (indicating higher risk) than seen in the earlier study by Mazumder *et al*. (1998) analyzed above.

The Von Ehrenstein et al. (2005) study of decrements in lung function related to arsenic exposure via drinking water reported slopes of -45.0 mL forced expiratory volume in 1 second (FEV_1) and -41.1 mL forced vital capacity (FVC) per 100 μg/L increase in arsenic concentration for exposed men. Assuming low dose linearity these values can be converted to inhalation values of 0.044 $\mu g/m^3$ (FEV_1) and 0.048 $\mu g/m^3$ (FVC) corresponding to respective 1 mL losses in lung function (e.g., 45/100 = 2.22 μg/L/mL decrement; 2.22 μg/L/mL x 2L water/d /(20m^3/d x 10UF x 0.5) = 0.044 $\mu g/m^3$/mL).

The inhalation values derived from oral human exposure studies above are summarized in Table 8.3.2. With the exception of the very low value derived from the pulse hypertension endpoint, the derived health protective inhalation values range over approximately forty fold from 0.044 to 1.7 $\mu g/m^3$. These adult values exceed the child-based values (range 0.015 to 1.6 $\mu g/m^3$). Therefore the proposed chronic REL value of 0.015 $\mu g/m^3$ is derived from the child arsenic exposure studies evaluated above and the adult studies provide supporting information.

Table 8.3.2 Inhalation Values Derived from Adult Human Drinking Water Studies

Study	Toxic Endpoint	Criterion	Value	Derived chronic REL, ($\mu g/m^3$)
Chiou *et al*. (1997b)	Cerebrovascular disease	LED_{01}	378 µg/d	1.26
Chiou *et al*. (1997b)	Cerebrovascular infarct	LED_{01}	332 µg/d	0.33
Chen *et al*. (1996a)	Ischemic Heart Disease Mortality	LED_{01}	5.53 (mg/L)-yr	0.16
Chen *et al*. (1995)	Hypertension	LED_{10}	5.8 (mg/L)-yr	0.55
Chen *et al*. (2006)	Systolic and pulse hypertension	SHT LED_{01} PHT LED_{10}	71.5 µg/L 0.49 µg/L	1.43 0.0033
Lai *et al*. (1994)	Diabetes mellitus	LED_{05}	8.8 (mg/L)-yr	0.85
Rahman *et al*. (1998)	Diabetes mellitus	LED_{05}	0.21 mg/L	1.4
Mazumder *et al*. (1998)	Skin keratosis	LED_{01}	50 µg/L	0.33
Rahman *et al*. (2006)	Skin keratosis or altered pigmentation	LED_{10}	65.4 µg/L	0.44
Tseng *et al*. (1996)	Peripheral vascular disease	est. LOAEL	20 (mg/L)-yr	1.69
Wang *et al*. (2002)	Carotid atherosclerosis	est. LOAEL	20 (mg/L)-yr	1.69
Von Ehrenstein *et al*. (2005)	Lung Function decrements	-1 mL FEV_1 -1 mL FVC	2.22 µg/L 2.42 µg/L	0.044 0.048

In addition to being inhaled, airborne arsenic can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for arsenic of 0.0035 µg/kg-day is also proposed. (From section 8.3.1.1, 2.3 µg/kg-d/(21.9 kg x 30UF) = 0.0035 µg/kg-d).

9. Arsine Based Calculations

The NAC/NRC (National Advisory Committee on Acute Exposure Guideline Levels for Hazardous Substances/National Research Council Subcommittee on Acute Exposure Guideline Levels) derived an Acute Exposure Guidance Level-2 (AEGL-2, disabling) of 0.17 ppm (500 $\mu g/m^3$) for one-hour exposure to arsine based on the hemolysis mouse data of Peterson and Bhattacharyya (1985) (Thomas and Young, 2001). Due to the steepness of the dose response the derivation of an AEGL-1 (Non-disabling) was considered inappropriate. Also the reliance on animal data was considered more "scientifically valid than AEGLs estimated from limited anecdotal human data". The panel used a total UF of 30 (10 for interspecies differences and 3 for intraspecies differences).

Based on the same study data, OEHHA calculated a continuous $BMDL_{1SD}$ of 2.17 ppm (6.9 mg/m^3) for reticulocytosis. When this value was adjusted with uncertainty factors of 10 for interspecies and 30 for intraspecies differences (including 10 for the intraspecies toxicokinetic sub-factor, as proposed in OEHHA, 2007 draft) the potential acute reference exposure level (aREL) for a one hour exposure was 2.17 ppm/300 = 0.0072 ppm (23 $\mu g/m^3$).

Despite the additional 10-fold margin of safety and more sensitive endpoint incorporated in the OEHHA derivation summarized above, there is still residual uncertainty in this comparison aREL value for arsine. There is particular concern with respect to the lack of adequate human data, given that rodents appear more resistant to the effects of acute exposure to various inorganic forms of arsenic than humans. The analogy between arsine and other inorganic forms of arsenic is supported by the observation that arsine exposure in humans and experimental animals results in similar metabolites excreted in urine as result from other inorganic arsenic exposure (Landrigan *et al*., 1982; Buchet *et al*., 1998). A further source of concern with a REL based on the Peterson and Bhattacharyya (1985) study is that while the margin of exposure for hemolysis is greater than 1000, the margin for total lethality is less than 4000. Although a steep dose-response slope for acute lethality is not unprecedented, it is a problematic feature when combined with the uncertainty in animal-to human extrapolation noted above. Thus, OEHHA staff have low confidence in using the Peterson and Bhattacharyya study as a basis of an aREL value for arsine and instead will rely on the aREL based on arsenic trioxide inhalation in mice (0.2 $\mu g/m^3$ arsenic, equivalent to 0.065 ppb arsine), which is sufficiently protective for all inorganic arsenic species.

A comparison of various possible values for an 8-hour REL for arsine is shown in Table 8.3.3. Adjustment of the one-hour NOAEL from Peterson and Bhattacharyya (1985) to eight hours using the modified Haber equation for mice gives a value of 1.6 ppm (4.98 mg/m^3)/300UF = 0.053 ppm (17 $\mu g/m^3$). This value is much higher than the values observed by Williams *et al*. (1981) in workers exposed to arsine concentrations estimated at 0.01 to 0.07 mg/m^3. The adverse effects noted included headache, nausea, weakness and vomiting. Although based on only a couple of subjects, the Williams *et al*. study would indicate an 8-hour value of about 0.04 mg/m^3/30 UF = 0.001 mg/m^3 or 1$\mu g/m^3$. Alternatively, the 90-day study of Blair *et al*. (1990) gives a NOAEL for hematologic effects in mice of 0.025 ppm arsine at 6 hours/day, 5 days/week. Applying the same 300 UF as above gives 0.083 ppb or 0.26 $\mu g/m^3$. This latter figure seems more in line with the limited human observations and more suitable for potentially

repeated 8-hour exposures to arsine. The intraspecies extrapolation includes additional uncertainty factors (PK + PD UF) for exposure of infants and children to arsine.

Table 8.3.3. Development of Health Protective Values for Arsine

Study	Toxic Endpoint	NOAEL/LOAEL/ BMDL	*Derived REL µg/m³*
Peterson and Bhattacharyya, 1985	Reticulocytosis in mice 1 hour exposure	$BMDL_{1SD}$ 2.17 ppm 6.9 mg/m^3	*Acute 23*
Peterson and Bhattacharyya, 1985	As above with 8-hour adjustment	1.6 ppm 4.98 mg/m^3	*8-hour 17*
Williams *et al*., 1981	Headache, nausea, weakness, and vomiting in exposed workers	0.01 to 0.07 mg/m^3, average 0.04 mg/m^3 LOAEL.	*8-hour 1.0*
Blair et al., *1990*	*Hematologic effects*	*NOAEL 0.025 ppm 6 hr/day*	*8-hour 0.26*

PBPK modeling of arsenic species in experimental animals and humans is presently considered inadequate to apply directly to the derivation of RELs for repeated arsine exposures.

Arsine exposure at atmospheric concentrations that caused adverse maternal effects did not adversely affect endpoints of developmental toxicity in mice or rats (Morrissey *et al*., 1990). In the absence of neurodevelopmental studies with arsine, it is assumed that such an effect would be comparable to those of other inorganic forms of arsenic. In view of the observed effect levels for hematological effects noted in the animal studies, both 8 hour and chronic effects of arsine are considered to be adequately covered by the respective cREL for inorganic arsenic based on neurodevelopmental effects observed in children (i.e., 0.015 $\mu g/m^3$ arsenic, equivalent to 0.005 ppb arsine)). In view of the concern over neurodevelopmental effects for all inorganic forms of arsenic, OEHHA concludes that it is appropriate to apply this value for 8-hour and chronic exposures to arsine.

10. Arsenic as a Toxic Air Contaminant that Disproportionately Impacts Children

In view of the neurodevelopmental toxicity studies discussed above, it is clear that infants and children are more susceptible to the toxicity of arsenic than adults. OEHHA recommends that inorganic arsenic and arsine be identified as a Toxic Air Contaminant the disproportionately impacts children under the California Health and Safety Code Section 39699.5.

11. References

ACGIH (1992). Documentation of the threshold limit values and biological exposure indices. Cincinnati (OH): American Conference of Government Industrial Hygienists, Inc.

Aposhian HV (1997). Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. Annu Rev Pharmacol Toxicol 37: 397-419.

Aposhian HV, Gurzau ES, Le XC, Gurzau A, Healy SM, Lu X, Ma M, Yip L, Zakharyan RA, Maiorino RM, Dart RC, Tircus MG, Gonzalez-Ramirez D, Morgan DL, Avram D and Aposhian MM (2000a). Occurrence of monomethylarsonous acid in urine of humans exposed to inorganic arsenic. Chem Res Toxicol 13(8): 693-7.

Aposhian HV, Zheng B, Aposhian MM, Le XC, Cebrian ME, Cullen W, Zakharyan RA, Ma M, Dart RC, Cheng Z, Andrewes P, Yip L, O'Malley GF, Maiorino RM, Van Voorhies W, Healy SM and Titcomb A (2000b). DMPS-arsenic challenge test. II. Modulation of arsenic species, including monomethylarsonous acid (MMA(III)), excreted in human urine. Toxicol Appl Pharmacol 165(1): 74-83.

Apostoli P, Alessio L, Romeo L, Buchet JP and Leone R (1997). Metabolism of arsenic after acute occupational arsine intoxication. J Toxicol Environ Health 52(4): 331-42.

Aranyi C, Bradof JN, Fenters JD, Graham JA and Miller FJ (1981). Effects of inhalation of arsenic trioxide aerosols in the pulmonary defenses of mice. In: International Conference. Heavy Metals in the Environment. Commission of the European Communities and the World Health Organization. 450-453.

Aranyi C, Bradof JN, O'Shea WJ, Graham JA and Miller FJ (1985). Effects of arsenic trioxide inhalation exposure on pulmonary antibacterial defenses in mice. J Toxicol Environ Health 15(1): 163-72.

ATSDR. (2000). *Arsenic. Toxicological Profile (Update)*. U.S. DHHS, Atlanta GA. http://www.atsdr.cdc.gov/toxprofiles/fp2-c4.pdf.

Baxley MN, Hood RD, Vedel GC, Harrison WP and Szczech GM (1981). Prenatal toxicity of orally administered sodium arsenite in mice. Bull Environ Contam Toxicol 26(6): 749-56.

Blair PC, Thompson MB, Bechtold M, Wilson RE, Moorman MP and Fowler BA (1990). Evidence for oxidative damage to red blood cells in mice induced by arsine gas. Toxicology 63(1): 25-34.

Blakley BR, Sisodia CS and Mukkur TK (1980). The effect of methylmercury, tetraethyl lead, and sodium arsenite on the humoral immune response in mice. Toxicol Appl Pharmacol 52(2): 245-54.

Blom S, Lagerkvist B and Linderholm H (1985). Arsenic exposure to smelter workers. Clinical and neurophysiological studies. Scand J Work Environ Health 11(4): 265-9.

Borgono JM, Vicent P, Venturino H and Infante A (1977). Arsenic in the drinking water of the city of Antofagasta: epidemiological and clinical study before and after the installation of a treatment plant. Environ Health Perspect 19: 103-5.

Brown MM, Rhyne BC and Goyer RA (1976). Intracellular effects of chronic arsenic administration on renal proximal tubule cells. J Toxicol Environ Health 1(3): 505-14.

Buchanan WD (1962). Arsine. In: Toxicity of Arsenic Compounds. Browning E. Elsevier. New York (NY): 67-75.

Buchet JP, Apostoli P and Lison D (1998). Arsenobetaine is not a major metabolite of arsine gas in the rat. Arch Toxicol 72(11): 706-10.

Buchet JP and Lauwerys R (1985). Study of inorganic arsenic methylation by rat liver in vitro: relevance for the interpretation of observations in man. Arch Toxicol 57(2): 125-9.

Buchet JP and Lauwerys R (1987). Study of factors influencing the in vivo methylation of inorganic arsenic in rats. Toxicol Appl Pharmacol 91(1): 65-74.

Buchet JP, Lauwerys R and Roels H (1981a). Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. Int Arch Occup Environ Health 48(1): 71-9.

Buchet JP, Lauwerys R and Roels H (1981b). Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. Int Arch Occup Environ Health 48(2): 111-8.

Calderon J, Navarro ME, Jimenez-Capdeville ME, Santos-Diaz MA, Golden A, Rodriguez-Leyva I, Borja-Aburto V and Diaz-Barriga F (2001). Exposure to arsenic and lead and neuropsychological development in Mexican children. Environ Res 85(2): 69-76.

Campbell D and Oates RK (1992). Childhood poisoning--a changing profile with scope for prevention. Med J Aust 156(4): 238-40.

Carter DE, Aposhian HV and Gandolfi AJ (2003). The metabolism of inorganic arsenic oxides, gallium arsenide, and arsine: a toxicochemical review. Toxicol Appl Pharmacol 193(3): 309-34.

Charbonneau SM, Spencer K, Bryce F and Sandi E (1978). Arsenic excretion by monkeys dosed with arsenic-containing fish or with inorganic arsenic. Bull Environ Contam Toxicol 20(4): 470-7.

Chen CJ, Chiou HY, Chiang MH, Lin LJ and Tai TY (1996). Dose-response relationship between ischemic heart disease mortality and long-term arsenic exposure. Arterioscler Thromb Vasc Biol 16(4): 504-10.

Chen CJ, Hsueh YM, Lai MS, Shyu MP, Chen SY, Wu MM, Kuo TL and Tai TY (1995). Increased prevalence of hypertension and long-term arsenic exposure. Hypertension 25(1): 53-60.

Chen CJ, Wu MM, Lee SS, Wang JD, Cheng SH and Wu HY (1988). Atherogenicity and carcinogenicity of high-arsenic artesian well water. Multiple risk factors and related malignant neoplasms of blackfoot disease. Arteriosclerosis 8(5): 452-60.

Chen KP and Wu HY (1962). Epidemiologic studies on blackfoot disease: II. A study of source of drinking water in relation to the disease. J Formosan Med Assoc 61: 611-618.

Chen Y, Factor-Litvak P, Howe GR, Graziano JH, Brant-Rauf P, Parvez F, Van Geen A, Ahsan H. (2006). Arsenic exposure from drinking water, dietary intakes of B vitamins and folate, and risk of high blood pressure in Bangladesh: A population-based, cross-sectional study. Am J Epidemiol 165:541-552.

Chi IC and Blackwell RQ (1968). A controlled retrospective study of blackfoot disease, an endemic peripheral gangrene disease in Taiwan. Am J Epidemiol 88: 7-24.

Chiou HY, Hsueh YM, Hsieh LL, Hsu LI, Hsu YH, Hsieh FI, Wei ML, Chen HC, Yang HT, Leu LC, Chu TH, Chen-Wu C, Yang MH and Chen CJ (1997a). Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. Mutat Res 386(3): 197-207.

Chiou HY, Huang WI, Su CL, Chang SF, Hsu YH and Chen CJ (1997b). Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 28(9): 1717-23.

Coles GA, Davies HJ, Daley D and Mallick NP (1969). Acute intravascular haemolysis and renal failure due to arsine poisoning. Postgrad Med J 45(521): 170-2.

Craig DK and Frye J. (1988). Acute LC50 nose only inhalation toxicity studies of arsine in rats (final report). N0512-4700. Batelle Columbus Labs. Columbus (OH)

Crump KS (1984). A new method for determining allowable daily intakes. Fundam Appl Toxicol 4(5): 854-71.

Crump KS and Howe R. (1983). Probit - A computer program to extrapolate quantile animal toxicological data to low doses. Crump K.S. & Company Inc.

Danielsson BR, Dencker L, Lindgren A and Tjalve H (1984). Accumulation of toxic metals in male reproduction organs. Arch Toxicol Suppl 7: 177-80.

DeSesso JM, Jacobson CF, Scialli AR, Farr CH and Holson JF (1998). An assessment of the developmental toxicity of inorganic arsenic. Reprod Toxicol 12(4): 385-433.

Engel RR and Smith AH (1994). Arsenic in drinking water and mortality from vascular disease: an ecologic analysis in 30 counties in the United States. Arch Environ Health 49(5): 418-27.

Ferm VH and Carpenter SJ (1968). Malformations induced by sodium arsenate. J Reprod Fertil 17(1): 199-201.

Flury F (1921). [Uber Kampfgasvergiftungen. IX. Lokal reizende Arsenverbindungen]. Zeichschrift fur die Gesamte Experimentelle Medizin 13: 527-528.

Flury F (1931). Schadliche Gase - dampfe, nebel, rauch - und staubarten. Berlin: Verlag von Julius Springer.

Fowler BA, Moorman MP, Adkins BJ, Bakewell WEJ, Blair PC and Thompson MB (1989). Arsine: toxicity data from acute and short-term inhalation exposures. In: ACGIH. Hazard assessment and control technology in semiconductor manufacturing. Lewis Publishers. Chelsea (MI): 85-89.

Frank G (1976). [Neurological and psychiatric disorders following acute arsine poisoning (author's transl)]. J Neurol 213(1): 59-70.

Freeman GB, Schoof RA, Ruby MV, Davis AO, Dill JA, Liao SC, Lapin CA and Bergstrom PD (1995). Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in cynomolgus monkeys. Fundam Appl Toxicol 28(2): 215-22.

Friberg L, Nordberg GF and Vouk VB, eds. (1986). Handbook on the Toxicology of Metals. Elsevier Amsterdam. p 59

Gates M, Williams J and Zapp JA. (1946). *Arsenicals. Summary technical report of Division 9, National Defense Research Committee. Vol. 1. Chemical warfare agents and related chemical problems.*: Office of Scientific Research and Development.

Gentry PR, Covington TR, Mann S, Shipp AM, Yager JW and Clewell HJ, 3rd (2004). Physiologically based pharmacokinetic modeling of arsenic in the mouse. J Toxicol Environ Health A 67(1): 43-71.

George B, Mathews V, Poonkuzhali B, Shaji RV, Srivastava A and Chandy M (2004). Treatment of children with newly diagnosed acute promyelocytic leukemia with arsenic trioxide: a single center experience. Leukemia 18(10): 1587-90.

Gladysheva TB, Oden KL and Rosen BP (1994). Properties of the arsenate reductase of plasmid R773. Biochemistry 33(23): 7288-93.

Grayson M, ed. (1978). Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley and Sons New York. 247-251

Gregus Z, Gyurasics A and Csanaky I (2000). Biliary and urinary excretion of inorganic arsenic: monomethylarsonous acid as a major biliary metabolite in rats. Toxicol Sci 56(1): 18-25.

Grobe VJ (1976). [Peripheral circulatory disorders and acrocyanosis in arsenic exposed Moselle wine-growers]. Berufsdermatosen 24(3): 78-84.

Hafeman DM, Ahsan H, Louis ED, Siddique AB, Slavkovich V, Cheng Z, van Geen A and Graziano JH (2005). Association between arsenic exposure and a measure of subclinical sensory neuropathy in Bangladesh. J Occup Environ Med 47(8): 778-84.

Hammamoto E (1955). [Infant arsenic poisoning by powdered milk]. Japanese Medical Journal 1649: 2-12 [cited in ATSDR, 2006].

Harrison WP and Hood RD (1981). Prenatal effecrs following exposure of hamsters to sodium arsenite by oral or intraperitoneal routes [abstract]. Teratology 23: 40A [cited in Willhite and Ferm, 1984].

Hatlelid KM, Brailsford C and Carter DE (1995). An in vitro model for arsine toxicity using isolated red blood cells. Fundam Appl Toxicol 25(2): 302-6.

Hatlelid KM, Brailsford C and Carter DE (1996). Reactions of arsine with hemoglobin. J Toxicol Environ Health 47(2): 145-57.

Hayakawa T, Kobayashi Y, Cui X and Hirano S (2005). A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19. Arch Toxicol 79(4): 183-91.

Healy SM, Casarez EA, Ayala-Fierro F and Aposhian H (1998). Enzymatic methylation of arsenic compounds. V. Arsenite methyltransferase activity in tissues of mice. Toxicol Appl Pharmacol 148(1): 65-70.

Heywood R and Sortwell RJ (1979). Arsenic intoxication in the rhesus monkey. Toxicol Lett 3: 137-144.

Hogue CJ, Buehler JW, Strauss LT and Smith JC (1987). Overview of the National Infant Mortality Surveillance (NIMS) project--design, methods, results. Public Health Rep 102(2): 126-38.

Holson JF, Stump DG, Ulrich CE and Farr CH (1999). Absence of prenatal developmental toxicity from inhaled arsenic trioxide in rats. Toxicol Sci 51(1): 87-97.

Hood RD and Harrison WP (1982). Effects of prenatal arsenite exposure in the hamster. Bull Environ Contam Toxicol 29(6): 671-8.

HSDB (1995). Hazardous Substances Data Bank. National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.

Hughes MF, Menache M and Thompson DJ (1994). Dose-dependent disposition of sodium arsenate in mice following acute oral exposure. Fundam Appl Toxicol 22(1): 80-9.

IARC. (2004). *Some Drinking -water Disinfectants and Contaminants, including Arsenic*. 84. International Agency for Research on Cancer, World Health Organization, Lyon, France.

ICRP (1994). Human Respiratory Tract Model for Radiological Protection. International Commission on Radiological Protection. Publication 66 Annals of the ICRP. Tarrytown (NY): Elsevier Science Inc. 24(1-3).

Ihrig MM, Shalat SL and Baynes C (1998). A hospital-based case-control study of stillbirths and environmental exposure to arsenic using an atmospheric dispersion model linked to a geographical information system. Epidemiology 9(3): 290-4.

IRDC. (1985). Three acute inhalation toxicity studies of arsine on rats (final reports). 533-001, 533-002, 533-003. International Research and Development Corporation. Mattawan (MI)

Itoh T, Zhang YF, Murai S, Saito H, Nagahama H, Miyate H, Saito Y and Abe E (1990). The effect of arsenic trioxide on brain monoamine metabolism and locomotor activity of mice. Toxicol Lett 54(2-3): 345-53.

Jacobson-Kram D and Montalbano D (1985). The reproductive effects assessment group's report on the mutagenicity of inorganic arsenic. Environ Mutagen 7(5): 787-804.

Ji G and Silver S (1992). Reduction of arsenate to arsenite by the ArsC protein of the arsenic resistance operon of Staphylococcus aureus plasmid pI258. Proc Natl Acad Sci U S A 89(20): 9474-8.

Jiang G, Gong Z, Li XF, Cullen WR and Le XC (2003). Interaction of trivalent arsenicals with metallothionein. Chem Res Toxicol 16(7): 873-80.

Kamil'dzhanov AX (1982). Hygiene basis for the maximum permissible concentration of arsenic trioxide in the ambient air. Gig Sanit 2: 74-75.

Kamkin AB (1982). [Review of the maximum permissible concentration of arsenic trioxide in the atmosphere of populated places]. Gig Sanit(1): 6-9.

Kitchin KT (2001). Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicol Appl Pharmacol 172(3): 249-61.

Kleinfeld MJ (1980). Arsine poisoning. J Occup Med 22(12): 820-1.

Klimecki WT and Carter DE (1995). Arsine toxicity: chemical and mechanistic implications. J Toxicol Environ Health 46(4): 399-409.

Krafft T and Macy JM (1998). Purification and characterization of the respiratory arsenate reductase of Chrysiogenes arsenatis. Eur J Biochem 255(3): 647-53.

Kurttio P, Komulainen H, Hakala E, Kahelin H and Pekkanen J (1998). Urinary excretion of arsenic species after exposure to arsenic present in drinking water. Arch Environ Contam Toxicol 34(3): 297-305.

Lagerkvist B, Linderholm H and Nordberg GF (1986). Vasospastic tendency and Raynaud's phenomenon in smelter workers exposed to arsenic. Environ Res 39(2): 465-74.

Lai MS, Hsueh YM, Chen CJ, Shyu MP, Chen SY, Kuo TL, Wu MM and Tai TY (1994). Ingested inorganic arsenic and prevalence of diabetes mellitus. Am J Epidemiol 139(5): 484-92.

Landrigan PJ, Costello RJ and Stringer WT (1982). Occupational exposure to arsine. An epidemiologic reappraisal of current standards. Scand J Work Environ Health 8(3): 169-77.

Le XC, Lu X, Ma M, Cullen WR, Aposhian HV and Zheng B (2000a). Speciation of key arsenic metabolic intermediates in human urine. Anal Chem 72(21): 5172-7.

Le XC, Ma M, Cullen WR, Aposhian HV, Lu X and Zheng B (2000b). Determination of monomethylarsonous acid, a key arsenic methylation intermediate, in human urine. Environ Health Perspect 108(11): 1015-8.

Levin-Scherz JK, Patrick JD, Weber FH and Garabedian C, Jr. (1987). Acute arsenic ingestion. Ann Emerg Med 16(6): 702-4.

Levvy GA (1947). A study of arsine poisoning. Quart J Exp Physiol 34: 47-67.

Lewis DR, Southwick JW, Ouellet-Hellstrom R, Rench J and Calderon RL (1999). Drinking water arsenic in Utah: A cohort mortality study. Environ Health Perspect 107(5): 359-65.

Lugo G, Cassady G and Palmisano P (1969). Acute maternal arsenic intoxication with neonatal death. Am J Dis Child 117(3): 328-30.

Lundgren KD (1954). [Damage to respiratory organs in workers in a smelting plant] cited in U.S. EPA, 1984. Nord Hyg Tidskr 3-4: 66-82.

Mann S, Droz PO and Vahter M (1994). A physiologically based pharmacokinetic model for four major arsenic species in mammals. In: Arsenic Exposure and Health Effects. Special Issue of Environmental Geochemistry and Health. Science and Technology Letters

Chappell W. R., Abernathy C. O. and Cothern C. R. Northwood. Middlesex, England: 16: 219-231.

Mann S, Droz PO and Vahter M (1996a). A physiologically based pharmacokinetic model for arsenic exposure. I. Development in hamsters and rabbits. Toxicol Appl Pharmacol 137(1): 8-22.

Mann S, Droz PO and Vahter M (1996b). A physiologically based pharmacokinetic model for arsenic exposure. II. Validation and application in humans. Toxicol Appl Pharmacol 140(2): 471-86.

Marafante E and Vahter M (1987). Solubility, retention, and metabolism of intratracheally and orally administered inorganic arsenic compounds in the hamster. Environ Res 42(1): 72-82.

Marnell LL, Garcia-Vargas GG, Chowdhury UK, Zakharyan RA, Walsh B, Avram MD, Kopplin MJ, Cebrian ME, Silbergeld EK and Aposhian HV (2003). Polymorphisms in the human monomethylarsonic acid (MMA V) reductase/hGSTO1 gene and changes in urinary arsenic profiles. Chem Res Toxicol 16(12): 1507-13.

Mazumder DNG, Haque R, Ghosh N, De BK, Santra A, Chakraborty D and Smith AH (1998). Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. Int J Epidemiol 27(5): 871-7.

Mazumder DN, Haque R, Ghosh N, De BK, Santra A, Chakraborti D, and Smith AH (2000). Arsenic in drinking water and the prevalence of respiratory effects in West Bengal, India. Int J Epidemiol 29(6):1047-1052.

Mazumder DN, Steinmaus C, Bhattacharya P, von Ehrenstein OS, Ghosh N, Gotway M, Sil A, Balmes JR, Haque R, Hira-Smith MM and Smith AH (2005). Bronchiectasis in persons with skin lesions resulting from arsenic in drinking water. Epidemiology 16(6): 760-765.

Menzel DB, Ross M, Oddo SV, Bergstrom PD, Greene H and Roth RN (1994). A physiologically based pharmacokinetic model for ingested arsenic. In: Arsenic Exposure and Health Effects. Special Issue of Environmental Geochemistry and Health. Science and Technology Letters. Chappell W. R., Abernathy C. O. and Cothern C. R. Northwood

Middlesex, England: 16: 209-218.

Meza MM, Yu L, Rodriguez YY, Guild M, Thompson D, Gandolfi AJ and Klimecki WT (2005). Developmentally restricted genetic determinants of human arsenic metabolism: association between urinary methylated arsenic and CYT19 polymorphisms in children. Environ Health Perspect 113(6): 775-81.

Morris JS, Schmid M, Newman S, Scheuer PJ and Sherlock S (1974). Arsenic and noncirrhotic portal hypertension. Gastroenterology 66(1): 86-94.

Morrissey RE, Fowler BA, Harris MW, Moorman MP, Jameson CW and Schwetz BA (1990). Arsine: absence of developmental toxicity in rats and mice. Fundam Appl Toxicol 15(2): 350-6.

Nagaraja TN and Desiraju T (1993). Regional alterations in the levels of brain biogenic amines, glutamate, GABA, and GAD activity due to chronic consumption of inorganic arsenic in developing and adult rats. Bull Environ Contam Toxicol 50(1): 100-7.

Nagaraja TN and Desiraju T (1994). Effects on operant learning and brain acetylcholine esterase activity in rats following chronic inorganic arsenic intake. Hum Exp Toxicol 13(5): 353-6.

Nagymajtenyi L, Selypes A and Berencsi G (1985). Chromosomal aberrations and fetotoxic effects of atmospheric arsenic exposure in mice. J Appl Toxicol 5(2): 61-3.

Nemec MD, Holson JF, Farr CH and Hood RD (1998). Developmental toxicity assessment of arsenic acid in mice and rabbits. Reprod Toxicol 12(6): 647-58.

Nordstrom S, Beckman L and Nordenson I (1979). Occupational and environmental risks in and around a smelter in northern Sweden. V. Spontaneous abortion among female employees and decreased birth weight in their offspring. Hereditas 90(2): 291-6.

NRC. (1984). *Emergency and Continuous Exposure Limits for Selected Airborne Contaminants.* . 1. National Academy Press, Washington, DC.

NRC. (2001). *Arsenic in Drinking Water 2001 Update*. National Academy Press, Washington, DC.

OEHHA. (1999). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part II: Technical Support Document for Describing Available Cancer Potency Factors*. Air Toxicology and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

OEHHA. (2000). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part IV: Technical Support Document for Exposure Assessment and Stochastic Analysis*. Air Toxicology and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/air/hot_spots/pdf/May2005Hotspots.pdf.

OEHHA. (2004). *Public Health Goals for Arsenic in Drinking Water*. Pesticide and Environmental Toxicology Branch, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/water/phg/allphgs.html.

OEHHA. (2007). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part V. Technical Support Document for the Derivation of Noncancer Reference Exposure Levels (5-15-07, draft).* Air Toxicology and Epidemiology Branch. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

Offergelt JA, Roels H, Buchet JP, Boeckx M and Lauwerys R (1992). Relation between airborne arsenic trioxide and urinary excretion of inorganic arsenic and its methylated metabolites. Br J Ind Med 49(6): 387-93.

Owen BA (1990). Literature-derived absorption coefficients for 39 chemicals via oral and inhalation routes of exposure. Regul Toxicol Pharmacol 11(3): 237-52.

Parish GG, Glass R and Kimbrough R (1979). Acute arsine posioning in two workers cleaning a clogged drain. Arch Environ Health 34(4): 224-7.

Perry K, Bowler RG, Bucknell HM, Druett HA and Scilling RSF (1948). Studies in the incidence of cancer in a factory handling inorganic compounds of arsenic. II. Clinical and environmental investigations. Br J Ind Med 5: 6-15.

Peterson DP (1990). Personal communication.

Peterson DP and Bhattacharyya MH (1985). Hematological responses to arsine exposure: quantitation of exposure response in mice. Fundam Appl Toxicol 5(3): 499-505.

Pomroy C, Charbonneau SM, McCullough RS and Tam GK (1980). Human retention studies with 74As. Toxicol Appl Pharmacol 53(3): 550-6.

Radabaugh TR and Aposhian HV (2000). Enzymatic reduction of arsenic compounds in mammalian systems: reduction of arsenate to arsenite by human liver arsenate reductase. Chem Res Toxicol 13(1): 26-30.

Rael LT, Ayala-Fierro F and Carter DE (2000). The effects of sulfur, thiol, and thiol inhibitor compounds on arsine-induced toxicity in the human erythrocyte membrane. Toxicol Sci 55(2): 468-77.

Rahman M and Axelson O (1995). Diabetes mellitus and arsenic exposure: a second look at case-control data from a Swedish copper smelter. Occup Environ Med 52(11): 773-4.

Rahman M, Tondel M, Ahmad SA and Axelson O (1998). Diabetes mellitus associated with arsenic exposure in Bangladesh. Am J Epidemiol 148(2): 198-203.

Rahman M, Tondel M, Ahmad SA, Chowdhury IA, Faruquee MH and Axelson O (1999). Hypertension and arsenic exposure in Bangladesh. Hypertension 33(1): 74-8.

Rahman M, Vahter M, Sohel N, Yunus M, Wahed MA, Streatfield PK, Ekstrom EC, Persson LA. (2006). Arsenic exposure and age- and sex-specific risk for skin lesions: A population-based case-referent study in Bangladesh. Environ Health Perspect 114:1847-1852.

Rahman MS, Hall LL and Hughes MF (1994). In vitro percutaneous absorption of sodium arsenate in B6C3F1 mice. Toxicol in Vitro 8(3): 441-448.

Rees DC and Hattis D (1994). Developing quantitative strategies for animal to human exprapolation. In: Principles and Methods of Toxicology. Hayes A. W. Raven Press. New York.

Rosenberg HG (1974). Systemic arterial disease and chronic arsenicism in infants. Arch Pathol 97(6): 360-5.

Rozenshtein IS (1970). [Sanitary toxicological assessment of low concentrations pf arsenic trioxide in the atmosphere]. Gig Sanit 35: 16-21.

Saha KC (2003). Saha's grading of arsenicosis progression and treatment. In: Arsenic Exposure and Health Effects V. Chappell W. R., Abernathy C. O. and Thomas D. J. Elsevier. San Diego: 391.

Sampayo-Reyes A, Zakharyan RA, Healy SM and Aposhian HV (2000). Monomethylarsonic acid reductase and monomethylarsonous acid in hamster tissue. Chem Res Toxicol 13(11): 1181-6.

Sanger DE (1987). Unpublished study cited in Blair *et al*., 1990. Amherst, MA: University of MassachusettsSchool of Public Health.

Schroeder HA and Mitchener M (1971). Toxic effects of trace elements on the reproduction of mice and rats. Arch Environ Health 23(2): 102-6.

Siripitayakunkit U, Lue S and Choprapawon C (2001). Possible effects of arsenic on visual perception and visual-motor integration of children in Thailand. In: Arsenic Exposure and Health Effects. Chappell W. R., Abernathy C. O. and Calderon R. L. Elsevier Science.

Siripitayakunkit U, Visudhiphan P, Pradipasen M and Vorapongsathron T (1999). Association between chronic arsenic exposure and children's intelligence in Thailand. In: Arsenic Exposure and Health Effects. Chappell W. R., Abernathy C. O. and Calderon J. Elsevier Science.

Smith AH, Goycolea M, Haque R and Biggs ML (1998). Marked increase in bladder and lung cancer mortality in a region of Northern Chile due to arsenic in drinking water. Am J Epidemiol 147(7): 660-9.

Stump DG, Holson JF, Fleeman TL, Nemec MD and Farr CH (1999). Comparative effects of single intraperitoneal or oral doses of sodium arsenate or arsenic trioxide during in utero development. Teratology 60(5): 283-91.

Styblo M, Delnomdedieu M and Thomas DJ (1996). Mono- and dimethylation of arsenic in rat liver cytosol in vitro. Chem Biol Interact 99(1-3): 147-64.

Styblo M, Yamauchi H and Thomas DJ (1995). Comparative in vitro methylation of trivalent and pentavalent arsenicals. Toxicol Appl Pharmacol 135(2): 172-8.

Tam GK, Charbonneau SM, Bryce F, Pomroy C and Sandi E (1979). Metabolism of inorganic arsenic (74As) in humans following oral ingestion. Toxicol Appl Pharmacol 50(2): 319-22.

Thomas R and Young R (2001). Arsine. Acute exposure guideline levels. Inhal Toxicol 13 (Suppl): 43-77.

Thompson DJ (1993). A chemical hypothesis for arsenic methylation in mammals. Chem Biol Interact 88(2-3): 89-14.

Tsai SY, Chou HY, The HW, Chen CM and Chen CJ (2003). The effects of chronic arsenic exposure from drinking water on the neurobehavioral development in adolescence. Neurotoxicology 24(4-5): 747-53.

Tseng CH, Chong CK, Chen CJ and Tai TY (1996). Dose-response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in blackfoot disease endemic villages in Taiwan. Atherosclerosis 120(1-2): 125-33.

Tseng WP (1977). Effects and dose--response relationships of skin cancer and blackfoot disease with arsenic. Environ Health Perspect 19: 109-19.

U.S. EPA. (2002). *Notice of Receipt of Requests to Cancel Certain Chromated Copper Arsenate (CCA) Wood Preservative Products and Amend to Terminate Certain Uses of CCA Products* OPP-66300; FRL-6826-8. U.S. Environmental Protection Agency.

U.S. EPA. (2006a). *EPA Chemical Profiles: Arsine*. U. S. Environmental Protection Agency. http://yosemite.epa.gov/oswer/ceppoehs.nsf/Profiles/1303-28-2?OpenDocument.

U.S. EPA. (2006b). *Organic herbicides (MSMA, DSMA, CAMA, and Cacodylic Acid), Registration Eligibility Decision: Notice of Availability.* Volume 71, No 153. Federal Register: August 9, 2006.

Vahter M (1981). Biotransformation of trivalent and pentavalent inorganic arsenic in mice and rats. Environ Res 25(2): 286-93.

Vahter M (1983). Metabolism of arsenic. In: Bilogical and Environmental Effects of Arsenic. Fowler B. A. Elsevier. Amsterdam: 171-198.

Vahter M, Concha G, Nermell B, Nilsson R, Dulout F and Natarajan AT (1995a). A unique metabolism of inorganic arsenic in native Andean women. Eur J Pharmacol 293(4): 455-62.

Vahter M, Couch R, Nermell B and Nilsson R (1995b). Lack of methylation of inorganic arsenic in the chimpanzee. Toxicol Appl Pharmacol 133(2): 262-8.

Vahter M and Envall J (1983). In vivo reduction of arsenate in mice and rabbits. Environ Res 32(1): 14-24.

Vahter M, Friberg L, Rahnster B, Nygren A and Nolinder P (1986). Airborne arsenic and urinary excretion of metabolites of inorganic arsenic among smelter workers. Int Arch Occup Environ Health 57(2): 79-91.

Vahter M, Marafante E and Dencker L (1984). Tissue distribution and retention of 74As-dimethylarsinic acid in mice and rats. Arch Environ Contam Toxicol 13(3): 259-64.

Vallee BL, Ulmer DD and Wacker WEC (1960). Arsenic toxicology and biochemistry. Arch Ind Health 21: 132-151.

von Ehrenstein OS, Guha Mazumder DN, Hira-Smith M, Ghosh N, Yuan Y, Windham G, Ghosh A, Haque R, Lahiri S, Kalman D, Das S and Smith AH (2006). Pregnancy outcomes, infant mortality, and arsenic in drinking water in West Bengal, India. Am J Epidemiol 163(7): 662-9.

von Ehrenstein OS, Mazumder DN, Yuan Y, Samanta S, Balmes J, Sil A, Ghosh N, Hira-Smith M, Haque R, Purushothamam R, Lahiri S, Das S and Smith AH (2005). Decrements in lung function related to arsenic in drinking water in West Bengal, India. Am J Epidemiol 162(6): 533-41.

Wagstaff DJ (1978). Alteration of hepatic detoxication enzyme activity by dietary arsenic trioxide. Food Cosmet Toxicol 16(5): 423-6.

Walton FS, Waters SB, Jolley SL, LeCluyse EL, Thomas DJ and Styblo M (2003). Selenium compounds modulate the activity of recombinant rat AsIII-methyltransferase and the methylation of arsenite by rat and human hepatocytes. Chem Res Toxicol 16(3): 261-5.

Wang CH, Jeng JS, Yip PK, Chen CL, Hsu LI, Hsueh YM, Chiou HY, Wu MM and Chen CJ (2002). Biological gradient between long-term arsenic exposure and carotid atherosclerosis. Circulation 105(15): 1804-9.

Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, van Geen A, Slavkovich V, LoIacono NJ, Cheng Z, Hussain I, Momotaj H and Graziano JH (2004). Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect 112(13): 1329-33.

Webb DR, Wilson SE and Carter DE (1986). Comparative pulmonary toxicity of gallium arsenide, gallium(III) oxide, or arsenic(III) oxide intratracheally instilled into rats. Toxicol Appl Pharmacol 82(3): 405-16.

Wester RC, Hui X, Barbadillo S, Maibach HI, Lowney YW, Schoof RA, Holm SE and Ruby MV (2004). In vivo percutaneous absorption of arsenic from water and CCA-treated wood residue. Toxicol Sci 79(2): 287-95.

Wester RC, Maibach HI, Sedik L, Melendres J and Wade M (1993). In vivo and in vitro percutaneous absorption and skin decontamination of arsenic from water and soil. Fundam Appl Toxicol 20(3): 336-40.

Wildfang E, Zakharyan RA and Aposhian HV (1998). Enzymatic methylation of arsenic compounds. VI. Characterization of hamster liver arsenite and methylarsonic acid methyltransferase activities in vitro. Toxicol Appl Pharmacol 152(2): 366-75.

Willhite CC and Ferm VH (1984). Prenatal and developmental toxicology of arsenicals. In: Nutritional and Toxiclogical Aspects of Food Safety. Friedman M. Plenum Publishing Corp. New York (NY): 9: 205-228.

Williams PL, Spain WH and Rubenstein M (1981). Suspected arsine poisoning during the restoration of a large cyclorama painting. Am Ind Hyg Assoc J 42(12): 911-3.

Winski SL and Carter DE (1995). Interactions of rat red blood cell sulfhydryls with arsenate and arsenite. J Toxicol Environ Health 46(3): 379-97.

Woods JS and Fowler BA (1978). Altered regulation of mammalian hepatic heme biosynthesis and urinary porphyrin excretion during prolonged exposure to sodium arsenate. Toxicol Appl Pharmacol 43(2): 361-71.

Wu MM, Kuo TL, Hwang YH and Chen CJ (1989). Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. Am J Epidemiol 130(6): 1123-32.

Yamauchi H and Yamamura Y (1985). Metabolism and excretion of orally administrated arsenic trioxide in the hamster. Toxicology 34(2): 113-21.

Yu D (1999). A physiologically based pharmacokinetic model of inorganic arsenic. Regul Toxicol Pharmacol 29(2 Pt 1): 128-41.

Yu HS, Sheu HM, Ko SS, Chiang LC, Chien CH, Lin SM, Tserng BR and Chen CS (1984). Studies on blackfoot disease and chronic arsenism in southern Taiwan: with special reference to skin lesions and fluorescent substances. J Dermatol 11(4): 361-70.

Yu L, Kalla K, Guthrie E, Vidrine A and Klimecki WT (2003). Genetic variation in genes associated with arsenic metabolism: glutathione S-transferase omega 1-1 and purine nucleoside phosphorylase polymorphisms in European and indigenous Americans. Environ Health Perspect 111(11): 1421-7.

Zakharyan R, Wu Y, Bogdan GM and Aposhian HV (1995). Enzymatic methylation of arsenic compounds: assay, partial purification, and properties of arsenite methyltransferase and monomethylarsonic acid methyltransferase of rabbit liver. Chem Res Toxicol 8(8): 1029-38.

Zakharyan RA and Aposhian HV (1999). Enzymatic reduction of arsenic compounds in mammalian systems: the rate-limiting enzyme of rabbit liver arsenic biotransformation is MMA(V) reductase. Chem Res Toxicol 12(12): 1278-83.

Zakharyan RA, Sampayo-Reyes A, Healy SM, Tsaprailis G, Board PG, Liebler DC and Aposhian HV (2001). Human monomethylarsonic acid (MMA(V)) reductase is a member of the glutathione-S-transferase superfamily. Chem Res Toxicol 14(8): 1051-7.

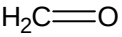
Zakharyan RA, Wildfang E and Aposhian HV (1996). Enzymatic methylation of arsenic compounds. III. The marmoset and tamarin, but not the rhesus, monkeys are deficient in methyltransferases that methylate inorganic arsenic. Toxicol Appl Pharmacol 140(1): 77-84.

Zaldivar R and Guillier A (1977). Environmental and clinical investigations on endemic chronic arsenic poisoning in infants and children. Zentralbl Bakteriol [Orig B] 165(2): 226-34.

Formaldehyde Reference Exposure Levels

(Methanal, oxomethane, methylene oxide)

CAS 50-00-0



1. Summary

The non-cancer adverse health effects of formaldehyde are largely a manifestation of its ability to irritate mucous membranes. As a result of its solubility in water and high reactivity, formaldehyde is efficiently absorbed into the mucus layers protecting the eyes and respiratory tract where it rapidly reacts, leading primarily to localized irritation. Acute high exposure may lead to eye, nose and throat irritation, and in the respiratory tract, nasal obstruction, pulmonary edema and dyspnea. Prolonged or repeated exposures have been associated with allergic sensitization, respiratory symptoms (coughing, wheezing, shortness of breath), histopathological changes in respiratory epithelium, and decrements in lung function. Children, especially those with diagnosed asthma, may be more likely to show impaired pulmonary function and symptoms than are adults following chronic exposure to formaldehyde. The studies reviewed for this document include those published through the Spring of 2008.

1.1 Formaldehyde Acute REL

Reference Exposure Level	**55 μg/m³ (44 ppb)**
Critical effect(s)	Mild and moderate eye irritation
Hazard Index target(s)	Eye irritation

1.2 Formaldehyde 8-Hour REL

Reference Exposure Level	**9 μg/m³ (7 ppb)**
Critical effect(s)	Nasal obstruction and discomfort, lower airway discomfort, and eye irritation
Hazard Index target(s)	Respiratory

1.3 Formaldehyde Chronic REL

Reference Exposure Level	**9 μg/m³ (7 ppb)**
Critical effect(s)	Nasal obstruction and discomfort, lower airway discomfort, and eye irritation
Hazard Index target(s)	Respiratory

2. Physical & Chemical Properties (ATSDR, 1999)

Description	Colorless gas
Molecular formula	CH_2O
Molecular weight	30.03 g/mol
Density	0.815 g/L @ -20° C
Boiling point	-19.5° C
Melting point	-92° C
Vapor pressure	3883 mm Hg @ 25° C
Flashpoint	300° C
Explosive limits	7% - 73%
Solubility	soluble in water, alcohol, ether and other polar solvents
Odor threshold	0.05-0.5 ppm
Metabolites	formic acid
Conversion factor	1 ppm in air = 1.24 mg/m^3 @ 25° C

3. Occurrence and Major Uses

Formaldehyde has four major applications: as an intermediate in the manufacture of melamine, polyacetal, and phenolic resins; as an intermediate in the production of industrial chemicals; as a bactericide or fungicide; and as a component in the manufacture of end-use consumer products. Phenol-formaldehyde resins are used in the production of plywood, particleboard, foam insulation, and a wide variety of molded or extruded plastic items. Formaldehyde is also used as a preservative, a hardening and reducing agent, a corrosion inhibitor, a sterilizing agent, and in embalming fluids. Indoor sources include upholstery, permanent press fabrics, carpets, pesticide formulations, urea-formaldehyde foam insulation, and cardboard and paper products. Outdoor sources include emissions from fuel combustion (motor vehicles), industrial fuel combustion (power generators), oil refining processes, and other uses (copper plating, incinerators, etc.). The largest portion of outdoor ambient formaldehyde results from photochemical oxidation of a number of reactive organic gases in the atmosphere (CARB, 2006). According to the California Toxics Inventory (CARB, 2005a), the mean statewide ambient level of formaldehyde in 2004 was 2.69 ppb, with the highest levels (3.76 ppb) reported for the South Coast Air Basin. The California Air Resources Board (CARB) reported statewide emissions of 20,251 tons from stationary and mobile sources (CARB, 2005b).

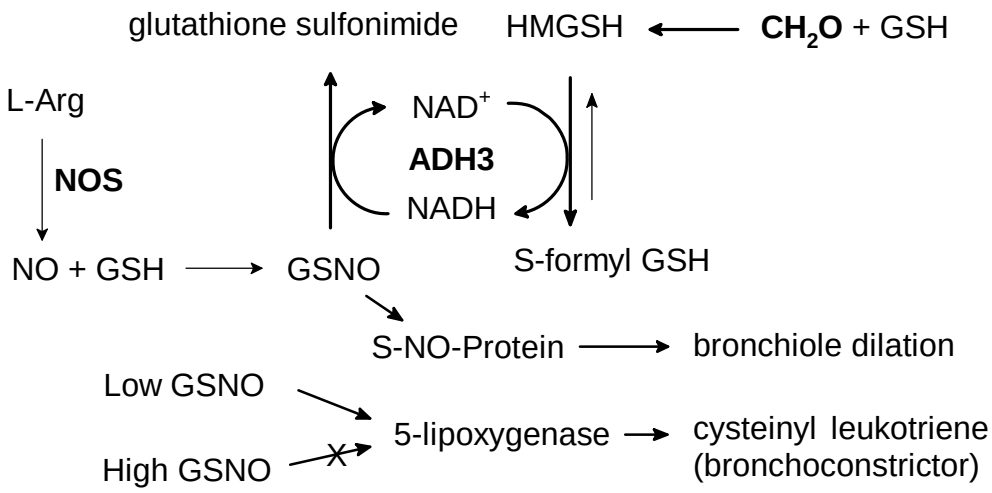
4. Metabolism

Inhaled formaldehyde reacts rapidly at the site of contact and is efficiently absorbed in the respiratory tract. A portion of the formaldehyde entering the fluid layer covering the respiratory epithelium, the respiratory tract lining fluid (RTLF), is reversibly hydrated to methylene glycol. Among other components, the RTLF is rich in antioxidants including glutathione (Cross et al., 1994) with which formaldehyde may reversibly react to form *S*-hydroxymethylglutathione. Both the hydrated and unreacted formaldehyde may be absorbed into the epithelial layer where there is further opportunity for formaldehyde to bind to glutathione. This glutathione conjugate in turn is oxidized to *S*-formylglutathione by formaldehyde dehydrogenase. Hydrolysis of *S*-formylglutathione yields formate and glutathione. Formic acid may be eliminated in urine and

feces, or dehydrogenated to CO_2 and exhaled. The presence of glutathione and formaldehyde dehydrogenase in epithelial cells of the respiratory tract varies with location and influences the amount of formaldehyde reaching the blood. While glutathione-bound formaldehyde is rapidly metabolized, free formaldehyde in cells can form DNA-protein cross-links (Franks, 2005).

Formaldehyde dehydrogenase (ADH3), although central to the metabolism of formaldehyde, has a broad specificity that includes the structurally related compound, S-nitrosoglutathione (GSNO), an endogenous bronchodilator and reservoir of nitric oxide (NO) activity (Jensen et al., 1998). In cultured cells, formaldehyde appears to trigger ADH3-mediated GSNO reduction by enzyme-bound cofactor recycling (Staab et al., 2008). As shown in Figure 1, the *S*-hydroxymethylglutathione (HMGSH) formed spontaneously from formaldehyde and glutathione is oxidized by ADH3 with the formation of NADH that may then participate in the ADH3-mediated reduction of GSNO (Thompson and Grafstrom, 2008). (Because of its participation in this reaction, ADH3 is also known as GSNO reductase.) This reductive pathway results in low levels of GSNO that in turn stimulate the production and activity of 5-lipoxygenase, the rate-limiting enzyme in the synthesis of powerful bronchoconstrictors, the cysteinyl leukotrienes. On the other hand, high levels of GSNO inhibit this enzyme and thus the synthesis of the bronchoconstrictors (Zaman et al., 2006). Up-regulation of the degradation of GSNO has been demonstrated in mouse lung following inhalation of formaldehyde (Yi et al., 2007), while low levels of GSNO in the lungs have been associated with severe asthma attacks in children (Gaston et al., 1998) and airway hyperactivity in mice (Que et al., 2005). These results suggest that the potential association of formaldehyde exposure with asthma-like respiratory symptoms is in part due to its effects on NO via the enhanced degradation of GSNO. Nitric oxide has multiple functions in the lungs, from its participation in the regulation of airway and vascular tone to mucin secretion and mucociliary clearance (Reynaert et al., 2005). The dysregulation of NO by formaldehyde helps to explain the variety and variability in the toxic manifestations following formaldehyde inhalation.

FIGURE 3 FORMALDEHYDE DRIVEN REDUCTION OF GSNO



Oxidation of the glutathione conjugate of formaldehyde, HMGSH, by ADH3 generates NADH that drives the reduction of GSNO, also by ADH3, thereby reducing the nitric oxide available for bronchiole dilation. Low GSNO levels stimulate, but high GSNO levels inhibit 5-lipoxygenase production of cysteinyl leukotriene.

5. Acute Toxicity of Formaldehyde

The acute effects of formaldehyde exposure appear to be largely a result of its irritant properties. However, some individuals experience symptoms following acute exposures that are a result of previous sensitization following acute high formaldehyde exposure, or long term low level exposures. For this reason, some of the studies included in this section describe manifestations of toxicity in which acute exposure was the precipitating event but in which the contribution of previous exposures or sensitization is unknown. Sensitization manifests as heightened responsiveness and may be of an immunological nature with the development of formaldehyde-specific IgE or IgG (e.g. Thrasher et al. 1987). Alternatively, heightened responsiveness may be neurologically mediated with involvement of the hypothalamic/pituitary/adrenal axis (Sorg et al., 2001a,b). In addition, genetic variation among individuals in the alcohol dehydrogenases mentioned above affects individual responses to formaldehyde. This is especially germane to studies in which the effects include symptoms such as bronchoconstriction and airway hyperreactivity, and in which there is unexpected individual variation.

Many of the studies described in this document have evaluated the relationship between formaldehyde inhalation and clinically-diagnosed asthma or asthma-like symptoms. Asthma is a chronic disease of airway obstruction resulting in variable airflow that has classically been considered to involve both airway inflammation and airway hyperresponsiveness. Asthma manifests as a characteristic cough, wheeze, and shortness of breath due to spasmodic contractions of the bronchi and mucus hypersecretion. These symptoms may or may not reflect an underlying allergic response. As shown in the study by Que et al. (2005), the hyperresponsiveness and the inflammation are not necessarily coupled. Although the RELs presented in this document are not based on studies that used asthma as the critical endpoint, uncertainty factors were applied in the REL estimates to explicitly consider the potential of

formaldehyde to cause or exacerbate asthma-like wheeze and cough symptoms, especially in asthmatic children. We have therefore included discussion of recent work that provides a biochemical mechanism by which formaldehyde exposure is linked to at least one symptom of asthma, bronchoconstriction. The bronchoconstrictive effects of formaldehyde exposure may be partially responsible for the lower airway discomfort reported in the study upon which the 8-hour and chronic RELs are based.

5.1 Acute Toxicity to Adult Humans

In small human studies, exposure to formaldehyde (1-3 ppm) has resulted in eye and upper respiratory tract irritation (Weber-Tschopp et al., 1977; Kulle et al., 1987). Most people cannot tolerate exposures to more than 5 ppm formaldehyde in air; above 10-20 ppm symptoms become severe and shortness of breath occurs (Feinman, 1988). High concentrations of formaldehyde may result in nasal obstruction, pulmonary edema, choking, dyspnea, and chest tightness (Porter, 1975; Solomons and Cochrane, 1984).

A few human case studies report severe pulmonary symptoms. A medical intern with known atopy and exposure to reportedly high (but unspecified) levels of formaldehyde over a period of 1 week developed dyspnea, chest tightness, and edema, following a subsequent 2 hour exposure to formaldehyde (Porter, 1975). Five workers exposed to formaldehyde from newly installed urea-formaldehyde chipboard in a poorly ventilated basement experienced intolerable eye and upper respiratory tract irritation, choking, marked dyspnea, and nasal obstruction (Solomons and Cochrane, 1984). However, the concentration of formaldehyde and the contribution of other airborne chemicals were unknown in both reports.

Numerous acute controlled and occupational human exposure studies have been conducted with both asthmatic and normal subjects to investigate formaldehyde's irritative and pulmonary effects (Frigas et al., 1984; Sheppard et al., 1984; Sauder et al., 1986; Schachter et al., 1986; Kulle et al., 1987; Sauder et al., 1987; Schachter et al., 1987; Witek et al., 1987; Uba et al., 1989; Harving et al., 1990; Akbar-Khanzadeh et al., 1994). Short exercise sessions during exposure on a bicycle ergometer were included in some of the studies. Concentrations of formaldehyde in the human exposure studies ranged as high as 3 ppm for up to 3 hours. The major findings in these studies were mild to moderate eye and upper respiratory tract irritation typical of mild discomfort from formaldehyde exposure.

Chemosensory irritation and subjective symptoms following exposure to formaldehyde at concentrations relevant to the workplace were examined by Lang et al. (2008) in 11 male and 10 female volunteers. Each subject was exposed for 4 hours to a randomized sequence of ten exposure conditions. These included exposures at concentrations of 0, 0.15, 0.3 and 0.5 ppm, exposures at 0.3 and 0.5 ppm that included four transient peak exposures at 0.6 and 1.0 ppm, respectively, and exposures in the presence of 10 ppm ethyl acetate of 0, 0.3, 0.5, and 0.5 ppm with 1.0 ppm peaks. Objective measures of irritation included conjunctival redness, blinking frequency, nasal flow resistance, pulmonary function, and reaction times. The participant's subjective evaluation of physical and mental wellbeing was assessed by questionnaire before, during and after each day's exposure. To assess the potential influence of personality traits on subjective responses, each subject's positive or negative affectivity was evaluated with PANAS (Positive and Negative Affectivity Schedule) that consists of 10 positive affects (interested,

excited, strong, enthusiastic, proud, alert, inspired, determined, attentive, and active) and 10 negative affects (distressed, upset, guilty, scared, hostile, irritable, ashamed, nervous, jittery, and afraid). Participants are asked to rate items on a scale from 1 to 5, based on the strength of emotion where 1 = "very slightly or not at all," and 5 = "extremely". Subjective ratings of eye irritation and olfactory symptoms were significantly higher than control at 0.3 ppm. However, when negative affectivity (anxiety) was included as a covariate, eye and olfactory irritation at this exposure level were no longer significant. Conjunctival irritation and blinking frequency, objective measures of irritation, were significantly elevated only with exposure to 0.5 ppm with peaks of 1.0 ppm ($p < 0.05$). The authors considered this level to be a LOAEL. However, at 0.5 ppm without 1.0 ppm peaks, conjuctival irritation and blinking were not significantly increased so this was considered a NOAEL for these effects. There were no statistically significant changes in nasal resistance, pulmonary function or reaction time. While there were large inter-individual differences in complaints or reports of wellbeing, there were no significant treatment effects. This study identified eye irritation as the most sensitive endpoint, with personality traits, such as negative affectivity, as a modifying factor.

In a human irritation study by Weber-Tschopp et al. (1977), 33 subjects were exposed to formaldehyde at concentrations ranging from 0.03-3.2 ppm (0.04-4.0 mg/m^3) for 35 minutes. Thresholds were 1.2 ppm (1.5 mg/m^3) for eye and nose irritation, 1.7 ppm (2.1 mg/m^3) for eye blinking, and 2.1 ppm (2.6 mg/m^3) for throat irritation.

Kulle et al. (1987) exposed nonasthmatic humans to up to 3.0 ppm (3.7 mg/m^3) formaldehyde in a controlled environmental chamber for 3 hours. Significant dose-response relationships were seen with odor and eye irritation (Table 5.1) as ranked on symptom questionnaires as none, mild, moderate or severe. Irritation was assessed in this manner prior to exposure, at the end of exposure, and again 24 hour after exposure.

TABLE 5.1 MEAN SYMPTOM DIFFERENCE (T_{180}-T_0) ± SE WITH FORMALDEHYDE* (FROM KULLE ET AL., 1987)

	Formaldehyde conc. (ppm)				P value
	0.0	1.0	2.0	3.0	
Odor sensation	0.00 ± 0.00	0.22 ± 0.15	0.44 ± 0.18	1.00 ± 0.29	<0.0001
Nose/throat irritation	0.00 ± 0.00	0.11 ± 0.11	0.33 ± 0.17	0.22 ± 0.15	0.054
Eye irritation	0.00 ± 0.00	0.44 ± 0.24	0.89 ± 0.26	1.44 ± 0.18	<0.0001
Chest discomfort	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.11	0.00 ± 0.00	0.62
Cough	0.00 ± 0.00	0.11 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.11
Headache	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.11	0.33

*Presence and severity of symptoms scored as: 0 = none; 1 = mild (present but not annoying); 2 = moderate (annoying); 3 = severe (debilitating). Data from Table II.

At 0.5 ppm for 3 hours, none of 9 subjects had eye irritation. At 1.0 ppm, 3 of 19 subjects reported mild eye irritation and one experienced moderate irritation. At 2.0 ppm, 6 subjects reported mild and 4 reported moderate eye irritation. Measured nasal flow resistance was increased at 3.0 ppm but not at 2.0 ppm (2.5 mg/m^3). With respect to the lower respiratory tract, there were no significant decrements in pulmonary function nor increases in methacholine

induced bronchial reactivity as a result of 3-hour exposures to 0.5-3.0 ppm (0.6-3.7 mg/m^3) formaldehyde at rest or during exercise, including 24 hours post exposure.

Eleven healthy subjects and nine patients with formalin skin sensitization were exposed to 0.5 mg/m^3 (0.4 ppm) formaldehyde for 2 hours (Pazdrak et al., 1993). Nasal lavage was performed prior to and 5 to 10 minutes, 4 hours, and 18 hours after exposure. Rhinitis was reported and increases in the number and proportion of eosinophils, elevated albumin and increased protein levels were noted in nasal lavage fluid 4 and 18 hours after exposure. No differences were found between patients with skin sensitization and healthy subjects.

In a study by Green et al. (1987), volunteer asthmatic and normal subjects exposed to formaldehyde displayed decrements in pulmonary function. Exposure to 3 ppm formaldehyde for 1 hour resulted in clinically significant reductions of forced expiratory volume in one second (FEV_1) (defined as > 20% or more) and FEV_1/forced vital capacity (FVC) (ratio 70% or less) in 5 individuals in the study (2 of 16 asthmatics, 2 of 22 normal subjects, and one clinically normal subject with hyperactive airways). Of these individuals, 3 had reductions of FEV_1 of 20% or more during exposure. One of 22 asthmatics had a greater than 20% reduction in FEV_1 (-25.8%) at 17 minutes into exposure following a 15 minute moderate exercise session (minute ventilation [V_E] = 30-40 l/min), which, according to the authors, was low enough to prevent exercise-induced bronchospasm. One of 22 normal subjects also exhibited a greater than 20% clinically significant reduction in FEV_1 (-24.4%) and in FEV_1/FVC, which occurred at 47 minutes into exposure to 3 ppm formaldehyde. These reductions occurred following a second 15- minute heavy-exercise session (V_E = 60-70 l/min) near the end of the 1 hour exposure period. A third asymptomatic "normal" subject with hyperactive airways had a clinically significant reduction of FEV_1 (-20.5%) at 17 minutes, following the first heavy exercise session. This subject exhibited occult airway hyperactivity and was excluded from analysis with the other exposure groups due to his respiratory condition. Subjects exhibiting reductions in FEV_1 of greater than 20% following exposure also exhibited FEV_1/FVC ratios of less than 70%. However, none of the subjects in the study exhibited a clinically significant reduction of 50% or greater in airway conductance (SG_{aw}) during exposure to 3 ppm formaldehyde.

Kriebel et al. (2001) conducted a subchronic epidemiological study of 38 anatomy class students who, on average, were exposed to a geometric mean of 0.70 ± 2.13 ppm for 2 hours per week over 14 weeks. After class, eye, nose and throat irritation was significantly elevated compared with pre-laboratory session exposures, with a one unit increase in symptom intensity/ppm of formaldehyde. Peak expiratory flow (PEF) was found to decrease by 1%/ppm formaldehyde during the most recent exposure. Changes in PEF and symptom intensity following formaldehyde exposure were most pronounced during the first weeks of the semester but attenuated with time, suggesting partial acclimatization.

Rhinitis and a wide range of respiratory symptoms can result from exposure to formaldehyde. Some studies have reported that workers exposed to low concentrations may develop severe prolonged asthma attacks after prior exposure; this suggests that they may have become sensitized (Feinman, 1988). However, in adults, an association between formaldehyde exposure and allergic sensitization through IgE- and IgG-mediated mechanisms has been observed only inconsistently (Thrasher et al., 1987; Krakowiak et al., 1998; Wantke et al., 2000; Kim et al., 2001).

Formaldehyde provocation of human subjects, occupationally exposed to formaldehyde and suffering from respiratory symptoms such as wheezing, shortness of breath, or rhinitis, occasionally resulted in pulmonary function decrements (2 to 33% response rate) consistent with immediate, delayed, or both immediate and delayed bronchoconstriction (Hendrick and Lane, 1977; Wallenstein et al., 1978; Burge et al., 1985; Nordman et al., 1985). While some of the concentrations of formaldehyde that elicited a positive response following provocation tests (6 to 20.7 ppm) were quite high, the authors of these studies suggested that formaldehyde-induced bronchial hyperreactivity is due to specific sensitization to the gas. However, none of these studies was able to detect antibodies to formaldehyde which would support that sensitization to formaldehyde occurs through an immunologic pathway. Alternatively, the wheezing and shortness of breath may be related to the formaldehyde-stimulated depletion of the bronchodilator, GSNO, in the airways.

In controlled studies with asthmatics from urea-formaldehyde insulated homes, formaldehyde concentrations equal to or greater than those found in indoor environments have not resulted in hematologic or immunologic abnormalities. These tests include: blood count and differential, erythrocyte sedimentation rate; lymphocyte subpopulations (E-rosetting, T3, T4, T8, B73.1, Fc receptor positive lymphocytes and large granular lymphocytes); lymphocyte response to phytohemagglutinin and formalin-treated red blood cells; serum antibody against the Thomsen-Friedenrich RBC antigen and against formalin-RBC; and natural killer, interferon-boosted natural killer, and antibody-dependent cell-mediated cytotoxicity (Pross et al., 1987). While six of the studies cited above reported decrements in lung function associated with short-term formaldehyde exposure among at least some of the asthmatic subjects, a number of other exposure studies of patients with asthma have failed to demonstrate that exposure to formaldehyde results in onset or aggravation of the patients' asthmatic symptoms (Sheppard et al., 1984; Sauder et al., 1987; Harving et al., 1990; Krakowiak et al., 1998).

The effects of formaldehyde on asthmatics may be dependent on previous, repeated exposure to formaldehyde. Burge et al. (1985) found that 3 out of 15 occupationally exposed workers challenged with formaldehyde vapors at concentrations from 1.5 ppm to 20.6 ppm for brief durations exhibited late asthmatic reactions. Six other subjects had immediate asthmatic reactions likely due to irritant effects. Asthmatic responses (decreased PEF, FVC, and FEV_1) were observed in 12 occupationally-exposed workers challenged with 2.0 ppm (2.5 mg/m^3) formaldehyde (Nordman et al., 1985). Similarly, asthmatic responses were observed in 5 of 28 hemodialysis workers occupationally exposed to formalin and challenged with formaldehyde vapors (concentration not measured) (Hendrick and Lane, 1977). In asthmatics not occupationally exposed to formaldehyde, Sheppard et al. (1984) found that a 10-minute challenge with 3 ppm formaldehyde coupled with moderate exercise did not induce significant changes in airway resistance or thoracic gas volume.

Gorski et al. (1992) evaluated the production of active oxygen species by neutrophils in 18 persons exposed to 0.5 mg/m^3 formaldehyde for 2 hours. All 13 subjects who had allergic contact dermatitis (tested positive to formaldehyde in skin patch) exhibited significantly higher chemiluminescence of granulocytes isolated from whole blood 30 minutes and 24 hours post-exposure than the individuals who were not formaldehyde sensitive. Thus, the immune cellular response of skin-sensitized individuals to an inhalation exposure to formaldehyde indicates increased production of active oxygen species. This is consistent with increasing evidence that

endogenous or exogenous reactive oxygen and reactive nitrogen species are responsible for the airway inflammation of asthma (Sugiura and Ichinose, 2008).

In addition to its effects on the respiratory tract, the irritant properties of formaldehyde also manifest as ocular irritation. In an anatomy dissecting laboratory, formaldehyde levels were found to peak at 0.62 ppm, with a gradual decrease to 0.11 ppm. Formaldehyde-related irritation of the eyes, nose, throat, airways and skin was reported by 59% of the students. These effects were significantly ($p < 0.001$) higher among wearers of contact lenses compared with students without glasses or wearing glasses (Tanaka et al., 2003). The ability of contact lenses to trap and concentrate volatile compounds, and to extend the exposure time by limiting the eye's normal self-cleansing, may make contact lens wearers more susceptible to ocular exposure and irritation by formaldehyde.

5.2 Acute Toxicity to Infants and Children

No studies of the effects of acute exposure to formaldehyde in children or young experimental animals were located. However, as noted above for adults, there is evidence that following acute exposure to formaldehyde, asthmatics and others previously sensitized to formaldehyde may be more likely to show respiratory symptoms such as wheezing, shortness of breath, rhinitis, and/or decrements in pulmonary function consistent with immediate and/or delayed bronchoconstriction (Nordman et al., 1985; Burge et al., 1985; Hendrick and Lane, 1977; Wallenstein et al., 1978). Furthermore, some asthmatics may respond with significant reductions in lung function due to the irritant effects on asthma, sensitized or not. Additionally, the depletion of the endogenous bronchodilator, GSNO, following formaldehyde exposure may be particularly important in children. Gaston et al. (1998) compared concentrations of tracheal S-nitrosothiol concentrations in eight asthmatic children in respiratory failure with those of 21 non-asthmatic children undergoing elective surgery. In asthmatic children, the metabolism of GSNO was accelerated and the mean S-nitrosothiol concentrations significantly lower compared to normal children (65 ± 45 vs 502 ± 429 nmol/l). Thus asthmatic children, with low levels of GSNO, are expected to be unusually vulnerable to any further depletion of GSNO caused by formaldehyde.

The potential association between formaldehyde exposure and asthma is of special concern for children since, as noted in OEHHA (2001): *"OEHHA considers asthma to impact children more than adults. Children have higher prevalence rates of asthma than do adults (Mannino et al., 1998). In addition, asthma episodes can be more severe due to the smaller airways of children, and result in more hospitalizations in children, particularly from the ages of 0 to 4 years, than in adults (Mannino et al., 1998)."* Thus children, particularly asthmatic children, may be at greater risk from acute exposure to formaldehyde.

5.3 Acute Toxicity to Experimental Animals

Acute exposures of experimental animals to formaldehyde are associated with changes in pulmonary function (decreased respiratory rate, increased airway reactivity and resistance) at low concentrations, while pulmonary edema and death have been reported at high concentrations. Neurochemical and neurobehavioral changes have also been observed.

In 72 rats exposed to approximately 600-1,700 mg/m^3 (500-1,400 ppm) formaldehyde vapor for 30 minutes, the LC_{50} was found to be 1,000 mg/m^3 (800 ppm) (Skog, 1950). The first deaths did not occur until 6 hours after cessation of exposure. Respiratory difficulty lasted several days after exposure and the last of 49 rats died after 15 days of purulent bronchitis and diffuse bronchopneumonia. Three weeks following exposure, histological examinations of the 23 surviving animals revealed bronchitis, pulmonary microhemorrhages, and edema. No changes were seen in other organs.

A multispecies study by Salem and Cullumbine (1960) showed that a 10-hour exposure to 15.4 ppm (19 mg/m^3) formaldehyde vapor killed 3 out of 5 rabbits, 8 of 20 guinea pigs, and 17 of 50 mice. The report stated that formaldehyde exposure resulted in delayed lethality.

Alarie (1981) determined the 10 minute LC_{50} for formaldehyde in mice to be 2,162 ppm (95% confidence interval, 1,687-2,770 ppm). The post-exposure observation period was 3 hours. From the concentration mortality graph provided in the report, an MLE_{05} and BC_{05} of 1,440 ppm and 778 ppm, respectively, could be estimated for a 10-minute formaldehyde exposure. However, as indicated in the previous reports, delayed deaths occur with formaldehyde which suggests that the 3-hour post-exposure observation period used in this study may not have been long enough.

In other lethality studies, Nagornyi et al.(1979) determined a 4-hour formaldehyde LC_{50} in rats and mice to be 588 mg/m^3 (474 ppm) and 505 mg/m^3 (407 ppm), respectively. However, the raw data for this study were not included in the report. Horton et al. (1963) observed that a 2-hour exposure of mice to 0.9 mg/l (900 mg/m^3) formaldehyde resulted in deaths from massive pulmonary hemorrhage and edema, but a 2 hour exposure to 0.14 mg/l (140 mg/m^3) did not produce signs of "substantial distress."

Swiecichowski et al., (1993) exposed groups of five to seven guinea pigs to 0.86, 3.4, 9.4, 31.1 ppm (1.1, 4.2, 11.6, 38.6 mg/m^3) formaldehyde for 2 hours, or to 0.11, 0.31, 0.59, 1.05 ppm (0.14, 0.38, 0.73, 1.30 mg/m^3) formaldehyde for 8 hours. An 8-hour exposure to levels greater than or equal to 0.3 ppm ($\geq$ 0.4 mg/m^3) formaldehyde was sufficient to produce a significant increase in airway reactivity. Similar effects occurred after greater than 9 ppm (> 11 mg/m^3) formaldehyde for the 2-hour exposure group. Formaldehyde exposure also heightened airway smooth muscle responsiveness to acetylcholine (or carbachol) *ex vivo*. No inflammation or epithelial damage was seen up to 4 days after exposure. The researchers suggest that duration of exposure is important to the induction of airway hyperreactivity and that prolonged (8-hour), low-level exposures may generate abnormal physiologic responses in the airways not detectable after acute (2-hour) exposures.

Male F-344 rats, 7-9 weeks old, were exposed to 0.5, 2, 6 or 15 ppm formaldehyde for 6 hours per day for 1 to 4 days (Monteiro-Riviere and Popp, 1986). Effects noted in the rat nasal respiratory epithelium with 0.5 or 2 ppm were limited to altered cilia with occasional wing-like projections on the ends of the ciliary shafts. Effects noted at 6 ppm for 1 day were autophagic vacuoles in some basal cells, neutrophils in the basal and suprabasal layers, and hypertrophy of goblet and ciliated cells. Loss of microvilli in ciliated cells was noted at all exposure concentrations.

Rats were exposed to 0, 5, 10 or 20 ppm formaldehyde for 3 hours per day on 2 consecutive days (Boja et al., 1985). Decreased motor activity and neurochemical changes in dopamine and 5-hydroxytryptamine neurons were reported.

The effects of formaldehyde inhalation on open-field behavior in mice were examined by Malek et al. (2004) 2 and 24 hours after a single 2-hour exposure to 0, 1.1, 2.3 or 5.2 ppm. Two hours after exposure there were significant decreases in rearing and in several measures of exploratory behavior, with evidence of dose-dependence in all dose groups compared with controls. At 24 hours, there were still significant differences between dosed and control mice but the dose-dependence was no longer evident.

Nielson et al. (1999) analyzed the breathing patterns of Balb/c mice exposed to 0.2-13 ppm formaldehyde and found a concentration-dependent decrease in respiratory rate of 32.9%/log concentration. In the range of 0.3-4.0 ppm, the decrease in respiratory rates was attributable to sensory irritation. Above 4.0 ppm, bronchoconstriction also contributed to the decreased breathing rate. The authors suggest a NOEL of 0.3 ppm for these effects in mice.

Amdur (1960) exposed groups of 4 to 18 guinea pigs to formaldehyde at 0.05, 0.31, 0.58, 1.22, 3.6, 11.0, or 49 ppm formaldehyde for one hour. Resistance to flow and lung compliance were calculated from measures of intrapleural pressure, tidal volume, and rate of flow to the lungs at the end of exposure and one hour later. Resistance and compliance were significantly different from the control level for the 0.31 ppm exposure ($p<0.05$) and increasingly significant at higher concentrations. One hour later, only the 49 ppm exposure remained significant ($p<0.01$). In addition, the tracheas of groups of 6 to 10 guinea pigs were cannulated and exposed for one hour to 0.90, 5.2, 20, or 50 ppm formaldehyde, and 1.14 or 3.6 ppm formaldehyde with 10 mg/m^3 sodium chloride. With the protective effect of the trachea bypassed, the resistance and compliance changed substantially. The addition of sodium chloride further enhanced the effect, including a significant effect after one hour for the 1.14 ppm formaldehyde exposure. These results show that formaldehyde that reaches the lungs has a marked effect on airways resistance and compliance in addition to an effect on the upper airways.

Riedel et al. (1996) studied the influence of formaldehyde exposure on allergic sensitization in guinea pigs. Three groups of guinea pigs (12/group) were exposed to clean air or two different formaldehyde concentrations (0.13 and 0.25 ppm) over five consecutive days. Following exposure, the animals were sensitized to allergen by inhalation of 0.5% ovalbumin (OA). Three weeks later the animals were subjected to bronchial provocation with OA and specific anti-OA-IgGl (reaginic) antibodies in serum were measured. In another group of six animals, the respiratory tract was examined histologically for signs of inflammation directly after the end of formaldehyde or clean air exposure. In the group exposed to 0.25 ppm formaldehyde, 10/12

animals were found to be sensitized to OA (positive reaction on specific provocation) vs. 3/12 animals in the control group ($P < 0.01$). Furthermore, compressed air measurements of specific bronchial provocation and serum anti-OA-antibodies were significantly higher in the 0.25 ppm formaldehyde group than in controls. The median for compressed air measurement was 0.35 ml for the formaldehyde-exposed group vs. 0.09 ml for the controls ($p< 0.01$), indicating increased bronchial obstruction. The median for the anti-OA-IgGl measured in the formaldehyde-exposed group was 13 vs. less than 10 EU in the controls, ($p < 0.05$), indicating enhanced sensitization. In the group exposed to 0.13 ppm formaldehyde, no significant difference was found compared to the control group. Histological examination found edema of the bronchial mucosa, but there was no sign of inflammation of the lower airways in formaldehyde-exposed guinea pigs. The investigators concluded that short-term exposure to a low concentration of formaldehyde (0.25 ppm) can significantly enhance sensitization to inhaled allergens in the guinea pig.

As described in Section 5, the main formaldehyde-metabolizing enzyme, ADH3, also reduces the endogenous bronchodilator GSNO. To examine the role of GSNO and ADH3 (known in this study as GSNO reductase, GSNOR) in airway tone and asthma, Que et al. (2005) used wild type mice and mice with a targeted deletion of GSNOR ($GSNOR^{-/-}$). Following a challenge with allergen (ovalbumin), GSNOR activity in bronchoalveolar lavage fluid from wild type mice increased significantly ($p < 0.05$) compared to buffer (PBS) controls, while as expected, no GSNOR activity was detected in the $GSNOR^{-/-}$ mice with either treatment. Levels of S-nitrosothiols (SNO) were assayed in homogenates of lung tissues from both types of mouse and found to be barely detectable with PBS treatment. However, after ovalbumin challenge, SNO levels were significantly higher ($p < 0.02$) in $GSNOR^{-/-}$ mice compared to wild type, indicating metabolism of SNOs by GSNOR under "asthmatic-like" conditions in wild type mice. Metabolism of GSNO results in a loss of bronchodilation capacity. Deletion of GSNOR had no effect on NO generation by NO synthase as there were no differences between wild type and $GSNOR^{-/-}$ mice in nitrate or nitrite levels regardless of treatment. To investigate the effects of deletion of GSNOR on airway hyper-responsivness, pulmonary resistance was measured at baseline (PBS) and after methacholine challenge, with and without ovalbumin treatment. At baseline, there was no difference among mouse types and treatments, while at higher methacholine levels (100-1000 µg/kg), pulmonary resistance was found to be significantly lower ($p < 0.001$) in $GSNOR^{-/-}$ mice than in wild type, presumably due to higher GSNO levels that enhance bronchodilation. Importantly, ovalbumin caused a marked increase in airway responsiveness in wild type mice but had little effect in $GSNOR^{-/-}$ mice. This indicates that GSNOR regulates basal airway tone as well as hyper-responsiveness to both allergen challenge and bronchoconstrictor agonists. It is also noteworthy that the total number and composition of leukocytes, levels of interleukin-13 and total serum IgE were comparable between wild type and $GSNOR^{-/-}$ mice. This indicates that protection from asthma in the $GSNOR^{-/-}$ mice is not a result of a suppressed response to allergen, and that SNOs, especially GSNO, can preserve airway patency in the presence of inflammation. Thus the inflammatory response is not linked to hyper-responsiveness as long as adequate levels of GSNO are maintained.

A connection between formaldehyde and the activity of GSNOR described in the study above by Que et al., was outlined by Thompson and Grafstrom (2008) and supported by Yi et al. (2007) and Staab et al. (2008). In the study by Yi and associates, groups of 6 mice were exposed to formaldehyde at 0, 1, or 3 mg/m^3 continuously for 72 hours. Following exposure, lungs were isolated to allow measurement of GSNOR mRNA levels by RT-PCR, and enzymatic activity

with GSNO. Formaldehyde at 3 mg/m^3 significantly increased the numbers of GSNOR transcripts compared to control (0.58 vs 0.4 GSNOR/ß actin; $p < 0.05$), while GSNOR reduction of GSNO showed a significant dose-dependent increase with formaldehyde concentration ($p < 0.01$). The stimulation of GSNO reduction by formaldehyde was also observed by Staab et al. (2008) in an in vitro study using recombinant human GSNOR. In this study, GSNO levels in buccal carcinoma cells were reduced in a dose-dependent fashion following a 1 hour exposure to formaldehyde in the 1-5 mM range with significance at 5 mM ($p < 0.05$). The results from this study support a model in which formaldehyde (as the glutathione conjugate, HMGSH) is oxidized by GSNOR (ADH3) in the presence of high levels of NAD^+, producing NADH. This process was found to be accelerated by high levels of GSNO. GSNO is in turn reduced with the oxidation of NADH to form glutathione sulfonimide. Formaldehyde thus depletes cellular SNO (in the form of GSNO) which results in dysregulation of NO signaling pathways.

6. Chronic Toxicity of Formaldehyde

6.1 Chronic Toxicity to Adult Humans

Formaldehyde primarily affects the mucous membranes of the upper airways and eyes. Exposed populations that have been studied include embalmers, residents in houses insulated with urea-formaldehyde foam, anatomy class students, histology technicians, wood and pulpmill workers, and asthmatics. A number of studies describing these effects have been briefly summarized below. For the sake of brevity, only the studies that best represent the given effects are presented. Formaldehyde is also a recognized carcinogen (IARC, 2006), however, this document will address only its non-carcinogenic properties.

In the study chosen for determination of the 8-hour and chronic RELs, nasal obstruction and discharge, and frequency of cough, wheezing, and symptoms of bronchitis were reported in 66 workers in a formaldehyde production plant exposed for 1-36 years (mean = 10 years) to a mean concentration of 0.21 ppm (0.26 mg/m^3) formaldehyde (Wilhelmsson and Holmstrom, 1992). All workers were exposed almost exclusively to formaldehyde, the concentrations of which were measured in the ambient air of the worksite with personal sampling equipment. Referents consisted of 36 office workers in a government office with exposure to a mean concentration of 0.07 ppm (0.09 mg/m^3) formaldehyde, and no industrial solvent or dust exposure. Symptom data, collected by questionnaire, were separated into general and work-related, and allowed identification of individuals with atopy and mucosal hyperreactivity. The critical effects from chronic exposure to formaldehyde in this study included nasal obstruction, lower airway discomfort, and eczema or itching. The frequency of reported lower airway discomfort (intermittent cough, wheezing, or symptoms of chronic bronchitis) was significantly higher among formaldehyde-exposed vs non-exposed workers (44 vs 14%; $p < 0.01$) (Table 6.1). Work-related nasal discomfort also was significantly higher in the formaldehyde group (53%) compared with the referent group (3%; $p < 0.001$). Similarly, work-related eye discomfort was 20% in the formaldehyde group but nonexistent among referents. The significant increase in symptoms of nasal discomfort in exposed workers did not correlate with total serum IgE antibody levels. However, two exposed workers, who complained of nasal discomfort, had elevated IgE levels. The investigators concluded that formaldehyde can induce nonspecific nasal hypersensitivity.

TABLE 6.1.1 SYMPTOMS OF FORMALDEHYDE EXPOSURE VS REFERENCE GROUP

(FROM WILHELMSSON AND HOLMSTROM, 1992)

	Formaldehyde	Reference	Rate difference	
	% (n=66)	% (n=36)	%	95% CI
General nasal discomfort	67	25	42	24-60
Workplace nasal discomfort	53	3	50	37-63
General lower airway discomfort	44	14	30	14-47
Workplace lower airway discomfort	33	3	28	15-40
General eye discomfort	24	6	18	6-36
General skin discomfort	36	11	25	10-41

In a cross-sectional study supportive of these results, Edling et al. (1988) reported histopathological changes in nasal mucosa of workers (n=75) occupationally exposed to formaldehyde (one wood laminating plant) or formaldehyde plus wood dust (two particle board plants). Ambient formaldehyde measurements in these three composite wood processing plants between 1975 and 1983 gave a time-weighted average (TWA) of 0.1-1.1 mg/m^3 (0.08- 0.89 ppm) with peaks of up to 5 mg/m^3 (4 ppm). The exposed workers were compared on the basis of medical and work histories, clinical examinations and nasal biopsies to 25 workers selected with regard to age and smoking habits but without occupational formaldehyde exposure.

Based on the histories, there was a high frequency of eye and upper airway symptoms among workers. Nasal symptoms (running nose and crusting) associated with formaldehyde exposure were reported in 60% of the workers, while 75% complained of lacrimation. Clinical examinations revealed grossly normal nasal mucosa in 75% of the cases while 25% had swollen or dry changes, or both, to the nasal mucosa. Histological examination (Table 6.2) revealed that only 3 of the 75 formaldehyde-exposed workers had normal, ciliated pseudostratified epithelium. Squamous metaplasia was reportedly observed in 59, while 6 showed mild dysplasia, and in 8 there was loss of ciliated cells and goblet cell hyperplasia. The histological grading showed a significantly higher score for nasal lesions among workers with formaldehyde exposure when compared with the referents (2.9 versus 1.8; $p < 0.05$). Exposed smokers had a higher, but non-significant, score than ex-smokers and non-smokers.

While the mean exposure time was 10.5 years (range 1-39 yr), there was no discernable difference among histology scores as a function of years of employment. The histology scores were also not different between workers in the particle board plants, exposed to both formaldehyde and wood dust, and workers in the laminate plant with exposure only to formaldehyde. The authors thus attribute the pathological changes in the nasal mucosa and the other adverse effects to formaldehyde alone in the 0.1-1.1 mg/m^3 range.

TABLE 6.1.2 DISTRIBUTION OF HISTOLOGICAL CHARACTERISTICS ASSOCIATED WITH FORMALDEHYDE EXPOSURE (FROM EDLING ET AL., 1988)

Histological characteristic	Grading score	Point score	Workers	%
Normal respiratory epithelium	0	0	3	4
Loss of ciliated cells	1	1	8	11
Mixed cuboidal/squamous epithelium, metaplasia	2	2	24	32
Stratified squamous epithelium	3	3	18	24
Keratosis	4	4	16	21
Budding of epithelium	add 1	5	0	0
Mild or moderate dysplasia	6	6	6	8
Severe dysplasia	7	7	0	0
Carcinoma	8	8	0	0

Histological changes in the nasal mucosa of formaldehyde-exposed workers were also reported by Boysen et al. (1990). In this study, nasal biopses were collected from 37 workers with 5 or more years of occupational formaldehyde exposure (0.5 - > 2 ppm) and compared with age-matched, unexposed controls who otherwise had similar environmental exposures and smoking habits. Histological changes in the nasal epithelium were scored as indicated in Table 6.1.3.

TABLE 6.1.3 TYPES OF NASAL EPITHELIA AND SCORING (FROM BOYSEN ET AL., 1990)

Types of epithelia	Histological score
Pseudostratified columnar	0
Stratified cuboidal	1
Mixed stratified cuboidal/stratified squamous	2
Stratified squamous, non-keratinizing	3
Stratified squamous, keratinizing	4
Dysplasia	5

As shown by the histological scoring in Table 6.1.4 below, metaplastic changes in the nasal epithelium were more pronounced in the formaldehyde-exposed workers although this difference did not reach statistical significance.

TABLE 6.1.4 HISTOLOGICAL SCORES OF NASAL EPITHELIA

		Histological score						
	No	0	1	2	3	4	5	Mean
Exposed	37	3	16	5	9	1	3	1.9
Controls	37	5	17	10	5	0	0	1.4

Rhinoscopical examination revealed hyperplastic nasal mucosa in 9 of 37 formaldehyde-exposed workers but in only 4 of the controls. In addition, the incidence of subjective nasal complaints was significantly ($p < 0.01$) higher in the exposed group. While the small size of this study, and the small amount of the nasal mucosa accessible to biopsy limited its ability to detect formaldehyde- related histopathology, the results are consistent with the histopathologies reported by Edling et al. above.

In another occupational health study (Grammer et al., 1990), 37 workers, who were exposed for an unspecified duration to formaldehyde concentrations in the range of 0.003 to 0.073 ppm, reported ocular irritation. However, no significant serum levels of IgE or IgG antibodies to formaldehyde-human serum albumin were detected.

Kerfoot and Mooney (1975) reported that estimated formaldehyde exposures of 0.25-1.39 ppm evoked numerous complaints of upper respiratory tract and eye irritation among seven embalmers at six different funeral homes. Three of the seven embalmers in this study reportedly had asthma. Levine et al. (1984) examined the death certificates of 1477 Ontario undertakers. Exposure measurements taken from a group of West Virginia embalmers were used as exposure estimates for the embalming process, ranging from 0.3-0.9 ppm (average 1-hour exposure) and 0.4-2.1 ppm (peak 30-minute exposure). Mortality due to non-malignant diseases was significantly elevated due to a two-fold excess of deaths related to the digestive system. The authors suggest increased alcoholism could have contributed to this increase.

Ritchie and Lehnen (1987) reported a dose-dependent increase in health complaints (eye and throat irritation, and headaches) in 2000 residents living in 397 mobile and 494 conventional homes. Complaints of symptoms of irritation were noted at concentrations of 0.1 ppm formaldehyde or above. Similarly, Liu et al. (1991) found that exposure to 0.09 ppm (0.135 mg/m^3) formaldehyde exacerbated chronic respiratory and allergy problems in residents living in mobile homes.

Employees of mobile day-care centers (66 subjects) reported increased incidence of eye, nose and throat irritation, unnatural thirst, headaches, abnormal tiredness, menstrual disorders, and increased use of analgesics as compared to control workers (Olsen and Dossing, 1982). The mean formaldehyde concentration in these mobile units was 0.29 ppm (0.43 mg/m^3) (range = 0.24 - 0.55 mg/m^3). The exposed workers were exposed in these units for a minimum of 3 months. A control group of 26 subjects in different institutions was exposed to a mean concentration of 0.05 ppm (0.08 mg/m^3) formaldehyde.

Occupants of houses insulated with urea-formaldehyde foam insulation (UFFI) (1726 subjects) were compared with control subjects (720 subjects) for subjective measures of irritation, measures of pulmonary function (FVC, FEV_1, FEF_{25-75}, FEF_{50}), nasal airway resistance, odor threshold for pyridine, nasal cytology, and hypersensitivity skin-patch testing (Broder et al., 1988). The mean length of time of exposure to UFFI was 4.6 years. The mean concentration of formaldehyde in the UFFI-exposed group was 0.043 ppm, compared with 0.035 ppm for the controls. A significant increase in symptoms of eye, nose and throat irritation was observed in subjects from UFFI homes, compared with controls. No other differences from control measurements were observed.

Alexandersson and Hedenstierna (1989) evaluated symptoms of irritation, spirometry, and immunoglobulin levels in 34 wood workers exposed to formaldehyde over a four-year period. Exposure to 0.4 - 0.5 ppm formaldehyde resulted in significant decreases in FVC, FEV_1, and FEF_{25-75}. Removal from exposure for four weeks allowed for normalization of lung function in the non-smokers.

Kriebel et al. (2001) conducted a subchronic epidemiological study of 38 anatomy class students who, on average, were exposed to a geometric mean of 0.70 ± 2.13 ppm formaldehyde for two hours per week over fourteen weeks. After class, eye, nose and throat irritation was significantly elevated compared with pre-laboratory session exposures, with a one unit increase in symptom intensity/ppm formaldehyde. Peak respiratory flow (PEF) was found to decrease by 1%/ppm formaldehyde during the most recent exposure. Changes in PEF and symptom intensity following formaldehyde exposure were most pronounced during the first week of the semester but attenuated with time, suggesting partial acclimatization.

Histology technicians (280 subjects) were shown to have reduced pulmonary function, as measured by FVC, FEV_1, FEF_{25-75}, and FEF_{75-85}, compared with 486 controls (Kilburn et al., 1989). The range of formaldehyde concentrations was 0.2 - 1.9 ppm, volatilized from formalin preservative solution.

Malaka and Kodama (1990) investigated the effects of formaldehyde exposure in plywood workers (93 exposed, 93 controls) exposed for 26.6 years, on average, to 1.13 ppm (range = 0.28 - 3.48 ppm). Fifty-three smokers were present in both exposed and control groups. Exposure assessment was divided into three categories: high (> 5 ppm), low (< 5 ppm), and none (reference group). Subjective irritation and pulmonary function tests were performed on each subject, and chest x-rays were taken of ten randomly selected volunteers from each group. Respiratory symptoms of irritation were found to be significantly increased in exposed individuals, compared with controls. In addition, exposed individuals exhibited significantly reduced FEV_1, FEV_1/FVC, and forced expiratory flow rate at 25% through 75% of FVC ($FEF_{25-75)}$, compared with controls. Forced vital capacity was not significantly reduced. Pulmonary function was not found to be different after a work shift, compared to the same measurement taken before the shift. No differences in chest x-rays were observed between exposed and control workers.

Occupational exposure to formaldehyde concentrations estimated to be 0.025 ppm (0.038 mg/m^3) for greater than six years resulted in complaints by 22 exposed workers of respiratory, gastrointestinal, musculoskeletal, and cardiovascular problems, and in elevated formic acid excretion in the urine (Srivastava et al., 1992). A control group of twenty seven workers unexposed to formaldehyde was used for comparison. A significantly higher incidence of abnormal chest x-rays was also observed in formaldehyde-exposed workers compared with controls.

Chemical plant workers (70 subjects) were exposed to a mean of 0.17 ppm (0.26 mg/m^3) formaldehyde for an unspecified duration (Holmstrom and Wilhelmsson, 1988). Compared with 36 control workers not exposed to formaldehyde, the exposed subjects exhibited a higher frequency of eye, nose, and deep airway discomfort. In addition, the exposed subjects had diminished olfactory ability, delayed mucociliary clearance, and decreased FVC.

Alexandersson et al. (1982) compared the irritant symptoms and pulmonary function of 47 carpentry workers exposed to a mean concentration of formaldehyde of 0.36 ppm (range = 0.04 - 1.25 ppm) with 20 unexposed controls. The average length of employment for the exposed workers was 5.9 years. Symptoms of eye and throat irritation as well as airway obstruction were more common in exposed workers. In addition, a significant reduction in FEV_1, FEV_1/FVC, and MMF was observed in exposed workers compared with controls.

Horvath et al. (1988) compared subjective irritation and pulmonary function in 109 workers exposed to formaldehyde with similar measures in a control group of 254 subjects. The formaldehyde concentrations for the exposed and control groups were 0.69 ppm (1.04 mg/m^3) and 0.05 ppm (0.08 mg/m^3), respectively. Mean formaldehyde concentration in the pre-shift testing facility and the state (Wisconsin) ambient outdoor - formaldehyde level were both 0.04 ppm (0.06 mg/m^3). Duration of formaldehyde exposure was not stated. Subjects were evaluated pre- and post work-shift and compared with control subjects. Significant differences in symptoms of irritation, FEV_1, FEV_1/FVC ratio, FEF_{50}, FEF_{25}, and FEF_{75} were found when comparing exposed subjects' pre- and post work-shift values. However, the pre-workshift values were not different from controls.

The binding of formaldehyde to endogenous proteins creates haptens that can elicit an immune response. Chronic exposure to formaldehyde has been associated with immunological hypersensitivity as measured by elevated circulating IgG and IgE autoantibodies to human serum albumin (Thrasher et al., 1987). In addition, a decrease in the proportion of T-cells was observed, indicating altered immunity. Thrasher et al. (1990) later found that long-term exposure to formaldehyde was associated with autoantibodies, immune activation, and formaldehyde-albumin adducts in patients occupationally exposed, or residents of mobile homes or of homes containing particleboard sub-flooring. The authors suggest that the hypersensitivity induced by formaldehyde may account for a mechanism for asthma and other health complaints associated with formaldehyde exposure.

An epidemiological study of the effects of formaldehyde on 367 textile and shoe manufacturing workers employed for a mean duration of 12 years showed no significant association between formaldehyde exposure, pulmonary function (FVC, FEV_1, and PEF) in normal or asthmatic workers, and occurrence of specific IgE antibodies to formaldehyde (Gorski and Krakowiak, 1991). The concentrations of formaldehyde did not exceed 0.5 ppm (0.75 mg/m^3).

Workers (38 total) exposed for a mean duration of 7.8 years to 0.11 - 2.12 ppm (mean = 0.33 ppm) formaldehyde were studied for their symptomatology, lung function, and total IgG and IgE levels in the serum (Alexandersson and Hedenstierna, 1988). The control group consisted of 18 unexposed individuals. Significant decrements in pulmonary function, FVC ($p < 0.01$) and FEV_1 ($p < 0.05$)) were observed, compared with the controls. Eye, nose, and throat irritation was also reported more frequently by the exposed group. No correlation was found between duration of exposure, or formaldehyde concentration, and the presence of IgE and IgG antibodies.

As described in section 5.1, chronic or repeated exposure to formaldehyde may influence the response of asthmatics to acute or short-term challenges. In the study by Burge et al. (1985) late asthmatic reactions were noted in 3 out of 15 occupationally exposed workers after short-duration exposure to 1.5 – 20.6 ppm formaldehyde. Similarly, among workers with occupational

exposure to formaldehyde, asthmatic responses (decreased PEF, FVC, and FEV_1) were reported in 12 workers challenged with 1.67 ppm (2.5 mg/m^3) formaldehyde (Nordman et al., 1985) and in 5 of 28 hemodialysis workers following challenge with formaldehyde vapors (concentration not measured) (Hendrick and Lane, 1977). In contrast, Sheppard et al. (1984) found that in asthmatics not occupationally exposed to formaldehyde, a 10-minute challenge with 3 ppm formaldehyde coupled with moderate exercise did not induce significant changes in airway resistance or thoracic gas volume. Thus individuals with chronic formaldehyde exposure may be at greater risk for adverse responses to acute exposures. These individuals may have been sensitized immunologically, as in the cases of elevated circulating antibodies, or rendered neurologically hyperresponsive, following repeated or chronic exposures to formaldehyde (Sorg et al., 2001a,b).

6.2 Chronic Toxicity to Infants and Children

There are few studies that compare the effects of chronic formaldehyde exposure on children versus adults. Among those that do there is evidence that children are more susceptible to the adverse effects of chronic exposure. Krzyzanowski et al. (1990) assessed chronic pulmonary symptoms and function in 298 children (6-15 years of age) and 613 adults (> 15 years of age) in relation to measured formaldehyde levels in their homes. Information on pulmonary symptoms and doctor-diagnosed asthma and chronic bronchitis was collected by questionnaire. Pulmonary function was assessed as peak expiratory flow rates (PEFR) measured up to four times a day. The prevalence of chronic respiratory symptoms in children was not related to formaldehyde levels measured in tertiles (< 40, 41-60, > 60 ppb). However, doctor-diagnosed asthma and chronic bronchitis were more prevalent in houses with elevated formaldehyde (p for trend < 0.02). This effect was driven by the high disease prevalence observed in homes with kitchen formaldehyde levels >60 ppb, and was especially pronounced among children with concomitant exposure to environmental tobacco smoke (Table 6.2.1). By comparison, in adults, while the prevalence rates of chronic cough and wheeze were somewhat higher in houses with higher formaldehyde, none of the respiratory symptoms or diseases was significantly related to formaldehyde levels.

TABLE 6.2.1 PREVALENCE RATE (PER 100) OF DIAGNOSED BRONCHITIS AND ASTHMA IN CHILDREN WITH FORMALDEHYDE (FROM KRZYZANOWSKI ET AL., 1990)

	Formaldehyde (ppb)			P value
Bronchitis	**≤ 40 (N)**	**41-60 (N)**	**>60 (N)**	**X^2 trend**
Household mean	3.5 (258)	17.2 (29)	9.1 (11)	<0.02
Main room mean	3.2 (253)	15.6 (32)	9.1 (11)	<0.01
Bedroom mean	3.8 (262)	16.0 (25)	9.1 (11)	<0.04
Subject's bedroom	4.7 (256)	6.7 (30)	11.1 (9)	>0.35
Kitchen	3.5 (255)	0 (22)	28.6 (21)	<0.001
No ETS	4.3 (141)	0 (12)	10.0 (10)	>0.40
ETS	1.9 (106)	0 (10)	45.5 (11)	<0.001
Asthma				
All children	11.7 (256)	4.2 (24)	23.8 (21)	<0.03
No ETS	8.5 (142)	8.3 (12)	0 (10)	>0.50
ETS	15.1 (106)	0 (12)	45.5 (11)	<0.05

In a random effects model, Krzyzanowski et al. (1990) reported that lung function (PEFR) in children, but not adults, was significantly decreased by formaldehyde (coefficient ± SE: -1.28 ± 0.46 vs 0.09 ± 0.27). Measurements of PEFR in the morning suggested that children with asthma (n = 4) were more severely affected than healthy children (coefficient ± SE: -1.45 ± 0.53 vs 0.09 ± 0.15) (Table 6.2.2). Compared to children, the effects of formaldehyde on pulmonary function in adults were smaller, transient, limited to morning measurements, and generally most pronounced among smokers exposed to the higher levels of formaldehyde. These studies suggest that children may be more susceptible to the effects of chronic formaldehyde exposure on lung function than are adults.

TABLE 6.2.2 RELATION OF PEFR (L/MIN) TO INDOOR FORMALDEHYDE

(from Krzyzanowski et al., 1990)

Factor	**Child coefficient ± SE**	**Adult coefficient ± SE**
HCHO house mean	-1.28 ± 0.46	0.09 ± 0.27
Morning vs bedtime	-6.10 ± 3.0	-5.90 ± 1.10
HCHO bdrm mean/morning	0.09 ± 0.15	-0.07 ± 0.04
HCHO bdrm mean/morning/asthma	-1.45 ± 0.53	

Among studies of children only, a case-control study by Rumchev et al. (2002) examined risk factors for asthma among young children (6 mo- 3 yr). Cases included children with clinically-diagnosed asthma, and controls were children of the same age group without such a diagnosis. Formaldehyde levels were measured in the homes, once in summer and once in winter. Questionnaires were used to assess potential risk factors for asthma and to collect parental reports of respiratory symptoms characteristic of asthma (cough, shortness of breath, wheeze, runny nose, trouble breathing, and hay fever) in their children. Formaldehyde levels were higher

in the homes of children exhibiting respiratory symptoms. Estimates of the relative risk for clinically-diagnosed asthma (odds ratios) were adjusted for measured indoor air pollutants, relative humidity, temperature, atopy, family history of asthma, age, gender, socioeconomic status, pets, smoke exposure, air conditioning, and gas appliances. Compared with children exposed to < 8 ppb, children in homes with formaldehyde levels > 49 ppb had a 39% higher risk of asthma ($p < 0.05$) after adjusting for common asthma risk factors.

Franklin et al. (2000) measured exhaled nitric oxide (eNO) levels in 224 children 6-13 years of age as an indicator of inflammation of the lower airways following chronic low-level formaldehyde exposure in the home. While there was no effect of formaldehyde on lung function measured by spirometry, eNO was significantly higher in children from homes with average formaldehyde levels ≥ 50 ppb compared with those from homes with levels ≤ 50 ppb (15.5 ppb eNO vs 8.7; $p = 0.02$).

Garrett et al. (1999) examined the association between formaldehyde levels at home (median 15.8 $\mu g/m^3$; maximum 139 $\mu g/m^3$) and atopy and allergic sensitization in 148 children, 7-14 years of age. The risk of atopy increased by 40% with each 10 $\mu g/m^3$ increase in bedroom formaldehyde. Two measures of allergic sensitization to twelve common environmental allergens, the number of positive skin prick tests and maximum wheal size, both showed linear associations with increasing maximum formaldehyde exposure levels. After adjusting for parental asthma and allergy, there was no evidence of an association between asthma in the children and formaldehyde levels. However, these data do suggest that formaldehyde levels commonly found in homes can enhance sensitization of children to common aeroallergens.

Of the numerous, primarily occupational, studies in adults, the NOAEL and LOAEL are 17 $\mu g/m^3$ (14 ppb) and 101 $\mu g/m^3$ (81 ppb), respectively, after adjustment for exposure continuity. These values are based on data on nasal and eye irritation as observed in Wilhelmsson and Holstrom (1992), and histological lesions in the nasal cavity as documented in Edling et al. (1988). However, studies in children, including the Krzyzanowski study above, indicate adverse health impacts in children at concentrations as low as 30 ppb. Wantke et al. (1996) reported that formaldehyde-specific IgE and respiratory symptoms were reduced when children transferred from schools with formaldehyde concentrations of 43 to 75 ppb to schools with concentrations of 23 to 29 ppb. While these human studies are not entirely consistent with each other, and there is potential for confounding in each, nevertheless, taken together, they suggest that children may be more sensitive to formaldehyde toxicity than adults.

A potential role for formaldehyde, GSNO and its metabolizing enzyme, GSNOR, in asthma is described in Section 5 above. The activity of GSNOR tends to be higher, and the levels of GSNO lower, in the lungs of asthmatics compared to non-asthmatics. This connection prompted Wu et al. (2007) to investigate whether genetic variation in GSNOR is associated with childhood asthma and atopy. The study group included 532 children, aged 4 to 17 with clinically diagnosed asthma, and their parents. Seven single nucleotide polymorphisms (SNPs) in GSNOR were genotyped in DNA extracted from lymphocytes to examine the relationship between common haplotypes and asthma. Atopy was determined with skin prick tests using a collection of 25 aeroallergens. Two of the GSNOR SNPs were associated with increased risk of asthma, but none was associated with atopy. Whereas a lower risk for asthma was associated with one (RR 0.77; 95% CI 0.61-0.97) or two (RR 0.66; 95% CI 0.44-0.99) copies of the minor A allele of

SNP rs1154404, homozygosity for the major T allele of this SNP carried an increased risk of asthma. Homozygosity for the minor allele of SNP re28730619 also carried an increased risk of asthma (RR 1.60; 95% CI 1.13-2.26; p = 0.0077). In the haplotype analysis, children with the most common GSNOR haplotype (GTCGG), that contained the major T allele of rs1154404 and the minor G allele of rs28730619, were at increased risk of childhood asthma. These results thus suggest that variants in GSNOR genotype influence childhood asthma susceptibility.

It should be noted that while term neonates have high levels of reduced glutathione in the fluid lining the lungs, these levels drop rapidly after birth. However, among premature infants, glutathione levels are typically substantially below those of term infants (Grigg et al., 1993) and adults (Reise et al., 1997). As a result of low levels of a critical component of formaldehyde metabolism, glutathione, these infants may be at increased risk from formaldehyde exposure.

6.3 Chronic Toxicity to Experimental Animals

Studies of the effects of chronic formaldehyde exposure in experimental animals tend to focus on lesions in the upper respiratory tract and the hyperplastic or metaplastic changes observed in the respiratory epithelium. Systemic effects, such as changes in body or organ weight, or blood chemistry, appear to be secondary to the effects of the olfactory irritation on feeding behavior. There is also evidence that repeated or long-term exposure to formaldehyde may cause neurologically-based hyperresponsiveness to formaldehyde (Sorg et al., 2001a) and altered expression of stress hormones (Sorg et al., 2001b).

In studies examining respiratory effects, Fischer-344 rats and B6C3F1 mice (120 animals/sex) were exposed to concentrations of 0, 2.0, 5.6, or 14.3 ppm formaldehyde vapor for 6 hours/day, 5 days/week for 24 months (Kerns et al., 1983). The exposure period was followed by up to six months of non-exposure. Interim sacrifices were conducted at 6, 12, 18, 24, 27, and 30 months. Both male and female rats in the 5.6 and 14.3 ppm groups demonstrated decreased body weights over the two-year period. At the 6 month sacrifice, the rats exposed to 14.3 ppm formaldehyde had non-neoplastic lesions of epithelial dysplasia in the nasal septum and turbinates. As the study progressed, epithelial dysplasia, squamous dysplasia, and mucopurulent rhinitis increased in severity and distribution in all exposure groups. In mice, cumulative survival decreased in males from 6 months to the end of the study. Serous rhinitis was detected at 6 months in the 14.3 ppm group of mice. Metaplastic and dysplastic changes were noted at 18 months in most rats in the 14.3 ppm group and in a few mice in the 5.6 ppm exposure group. By 24 months, the majority of mice in the 14.3 ppm group had metaplastic and dysplastic changes associated with serous rhinitis, in contrast to a few mice in the 5.6 ppm group and a few in the 2 ppm group (exact number not given).

Woutersen et al. (1989) exposed male Wistar rats (60 animals/group) 6 hours/day for 5 days/week to 0, 0.1, 1.0 and 10 ppm formaldehyde vapor for 28 months. Compound-related nasal lesions of the respiratory and olfactory epithelium were observed only in the 10 ppm group. In the respiratory epithelium, the lesions consisted of rhinitis, squamous metaplasia and basal cell/pseudoepithelial hyperplasia. In the olfactory region, the lesions included epithelial degeneration and rhinitis. No differences in behavior or mortality were noted among the various groups. However, growth retardation was observed in the 10 ppm group from day 14 onwards. In a parallel study, male Wistar rats were exposed to 0, 0.1, 1.0 and 10 ppm formaldehyde for 3

months followed by a 25-month observation period. Compound-related histopathological changes were found only in the noses of the 10 ppm group and comprised increased incidence of squamous metaplasia of the respiratory epithelium, and rhinitis.

In a chronic exposure study that primarily investigated aspects of nasal tumor development, Monticello et al. (1996) examined nasal cavities of male F-344 rats (0-10 ppm, 90 animals/group; 15 ppm, 147 animals) following exposure to 0, 0.7, 2, 6, 10, and 15 ppm formaldehyde for 6 hours/day, 5 days/week for 24 months. Treatment-related decreases in survival were apparent only in the 15 ppm group. Nasal lesions at the two highest doses included epithelial hypertrophy and hyperplasia, squamous metaplasia, and a mixed inflammatory cell infiltrate. Lesions in the 6 ppm group were minimal to absent and limited to focal squamous metaplasia in the anterior regions of the nasal cavity. No formaldehyde-induced lesions were observed in the 0.7 or 2 ppm groups.

Kamata et al. (1997) exposed 32 male F-344 rats/group to gaseous formaldehyde at 0, 0.3, 2, and 15 ppm 6 hours/day, 5 days/week for up to 28 weeks. A room control, non-exposed group was also included in the study. Five animals per group were randomly selected at the end of the 12, 18, and 24 months, and surviving animals at 28 months were sacrificed for full pathological evaluation. Behavioral effects related to sensory irritation were evident in the 15 ppm group. Significant decreases in food consumption, body weight and survival were also evident in this group. No exposure-related hematological findings were observed. Biochemical and organ weight examination revealed decreased triglyceride levels and absolute liver weights at the highest exposure, but was likely related to reduced food consumption. Abnormal histopathological findings were confined to the nasal cavity. Inflammatory cell infiltration, erosion or edema of the nasal cavity was evident in all groups, including controls. Significantly increased incidence of non-proliferative (squamous cell metaplasia without epithelial cell hyperplasia) and proliferative lesions (epithelial cell hyperplasia with squamous cell metaplasia) were observed in the nasal cavities beginning at 2 ppm. In the 0.3 ppm group, a non-significant increase in proliferative nasal lesions (4/20 animals) were observed in rats that were either sacrificed or died following the 18^{th} month of exposure.

Rusch et al. (1983) exposed groups of 6 male cynomolgus monkeys, 20 male or female rats, and 10 male or female hamsters to 0, 0.2, 1.0, or 3.0 ppm (0, 0.24, 1.2, or 3.7 mg/m^3) formaldehyde vapor for 22 hours/day, 7 days/week for 26 weeks. There was no treatment-related mortality during the study. In monkeys, the most significant findings were hoarseness, congestion and squamous metaplasia of the nasal turbinates in 6/6 monkeys exposed to 2.95 ppm. There were no signs of toxicity in the lower exposure groups. In the rat, squamous metaplasia and basal cell hyperplasia of the nasal epithelia were significantly increased in rats exposed to 2.95 ppm. The same group exhibited decreased body weights and decreased liver weights. In contrast to monkeys and rats, hamsters did not show any signs of response to exposure, even at 2.95 ppm.

Kimbell et al. (1997) exposed male F-344 rats ($\leq$ 6/group) to 0, 0.7, 2, 6, 10, and 15 ppm 6 hr/day, 5 days/week for 6 months. Squamous metaplasia was not observed in any regions of the nasal cavity in any of the control, 0.7, or 2 ppm groups. However, the extent and incidence of squamous metaplasia in the nasal cavity increased with increasing dose beginning at 6 ppm.

In subchronic studies, Wilmer et al. (1989) found that intermittent (8 hours/day, 5 days/week) exposures of rats to 4 ppm formaldehyde for 13 weeks resulted in significant histological changes in the nasal septum and turbinates. In contrast, continuous exposure of rats for 13 weeks to 2 ppm formaldehyde did not produce significant lesions. This study revealed the concentration dependent nature of the nasal lesions caused by formaldehyde exposure. Zwart et al. (1988) exposed male and female Wistar rats (50 animals/group/sex) to 0, 0.3, 1, and 3 ppm formaldehyde vapor for 6 hr/day, 5 days/week for 13 weeks. Compound related histopathological nasal changes varying from epithelial disarrangement to epithelial hyperplasia and squamous metaplasia were found in the 3 ppm group, and were restricted to a small area of the anterior respiratory epithelium. These changes were confirmed by electron microscopy and were not observed in other groups.

Woutersen et al. (1989) exposed rats (20 per group) to 0, 1, 10, or 20 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks. Rats exposed to 20 ppm displayed retarded growth, yellowing of the fur, and significant histological lesions in the respiratory epithelium. Exposure to 10 ppm did not affect growth, but resulted in significant histological lesions in the respiratory tract. No effects on specific organ weights, blood chemistries, liver glutathione levels, or urinalysis were detected at any level. No significant adverse effects were seen at the 1.0 ppm exposure level.

Appelman et al. (1988) found significant nasal lesions in rats (20 per group; 0, 0.1, 1.0, or 10.0 ppm) exposed to 10 ppm formaldehyde 6 hours/day, 5 days/week for 52 weeks, but exposure to 1.0 ppm or less for this period did not result in nasal histological lesions. However, the rats exposed to formaldehyde displayed decreased body weight in all groups compared with controls.

Apfelbach and Weiler (1991) determined that rats (5 exposed, 10 controls) exposed to 0.25 ppm (0.38 mg/m^3) formaldehyde for 130 days lost the olfactory ability to detect ethyl acetate odor.

Maronpot et al. (1986) exposed groups of 20 mice to 0, 2, 4, 10, 20, or 40 ppm formaldehyde 6 hours/day, 5 days/week, for 13 weeks. Histological lesions in the upper respiratory epithelium were seen in animals exposed to 10 ppm or greater. Exposure to 40 ppm was lethal to the mice.

A six-month exposure of rats to 0, 0.5, 3, and 15 ppm formaldehyde (3 rats per group) resulted in significantly elevated total lung cytochrome P450 in all formaldehyde-exposed groups (Dallas et al., 1989). The degree of P450 induction was highest after 4 days exposure and decreased slightly over the course of the experiment.

A series of studies have addressed the effects of long-term repeated exposures to formaldehyde on altered functioning of the hypothalamic-pituitary-adrenal (HPA) axis (Sorg et al., 2001b) and on neurobehavioral changes in rats (Sorg et al., 2001a). To study formaldehyde's effects on the HPA, Sorg et al. (2001b) measured corticosterone levels in the trunk blood of male Sprague-Dawley rats 20 or 60 min following acute chamber exposures to air or formaldehyde (0.7 or 2.4 ppm). All groups showed increased corticosterone levels above naive basal levels at 20 min followed by a return to baseline by 60 min, with no differences between treatment groups. A second experiment assessed the effects of repeated formaldehyde exposure (1 h/day, 5 days/week for 2 or 4 weeks) on basal corticosterone levels and those after a final challenge. Basal corticosterone levels were increased above naive values after 2 week exposure to air or 0.7 ppm

formaldehyde. By 4 weeks, corticosterone levels in the air group returned to naive values, but remained elevated in the 0.7 ppm formaldehyde group. There were no differences in basal corticosterone levels among either 2.4 ppm exposed groups. After a final air or formaldehyde challenge, the 2 and 4 week air and 0.7 ppm formaldehyde groups had elevated corticosterone levels similar to their acute response, while in the 2 and 4 week 2.4 ppm formaldehyde groups, corticosterone levels were higher than their acute response levels, indicating enhanced reactivity of the HPA axis to subsequent formaldehyde. It thus appears that repeated low-level formaldehyde exposure alters HPA axis functioning and the release of stress hormones. Since glucocorticoids may stimulate or inhibit the synthesis of surfactant-associated proteins in the lung (Liley et al., 1988), the alteration of HPA function may represent another pathway by which formaldehyde affects pulmonary function. For example, the pulmonary surfactants that regulate surface tension in the lungs are in turn regulated by surfactant-associated proteins. Reports of lower airway discomfort associated with chronic formaldehyde exposure may be related to the altered release or activity of these surfactant-associated proteins in the lung.

In another study of the effects of formaldehyde and the hypothalamus-pituitary-adrenal (HPA) axis, Sari et al. (2004) exposed female C3H/He mice to formaldehyde (0, 80, 400, 2000 ppb) by inhalation for 16 h/day, 5 days/week, for 12 weeks. Immunocytochemistry was used to examine corticotropin releasing hormone (CRH)-immunoreactive (ir) neurons in the hypothalamus, and adrenocorticotropin hormone (ACTH)-ir cells in the pituitary. RT-PCR was used to quantify ACTH rnRNA in the pituitary. Two groups of female mice were exposed, one of which comprised control mice with no allergen exposure. The other group was made allergic by injection of ovalbumin and alum prior to exposure to formaldehyde. Animals in the second group were further exposed to aerosolized ovalbumin as a booster four times during the exposure period. In the non-allergic group, formaldehyde caused a dose-dependent increase in the number of CRH-ir neurons with a similar pattern of increases in ACTHir cells and ACTH mRNA. The allergic mice showed an increase in basal levels of all these markers of HPA activity, and were responsive to the lowest concentration of formaldehyde. Thus at low levels of exposure, allergen and formaldehyde exposure exacerbate each other's effects on the stress response of the HPA.

7. Developmental and Reproductive Toxicity

In humans there are few data on the association of teratogenicity or adverse reproductive effects with formaldehyde exposure. Existing data do not suggest that formaldehyde, by inhalation or oral routes, produces significant teratogenic or reproductive effects (ATSDR, 1999).

A developmental toxicity study on formaldehyde was conducted by Martin (1990). Pregnant rats (25 per group) were exposed to 0, 2, 5, or 10 ppm formaldehyde for 6 hours/day, during days 6-15 of gestation. Although exposure to 10 ppm formaldehyde resulted in reduced food consumption and body weight gain in the maternal rats, no effects on the number, viability or normal development of the fetuses were seen. In addition, Saillenfait et al. (1989) exposed pregnant rats (25 per group) to 0, 5, 10, 20, or 40 ppm formaldehyde from days 6 - 20 of gestation. Maternal weight gain and fetal weight were significantly reduced in the 40 ppm exposure group. No significant fetotoxicity or teratogenic defects were observed at formaldehyde levels that were not also maternally toxic.

Evidence of embryotoxicity was reported by Kitaeva et al. (1990) in embryos of rats that had been exposed to formaldehyde by inhalation 4 h/d, 5 d/wk for 4 months. At 1.5 mg/m^3, but not at 0.5 mg/m^3, there was a significant increase in the proportion of degenerate embryos. By comparison, the bone marrow cells of the mothers appeared to be more sensitive to formaldehyde as shown by significant increases in the numbers of cells with aberrations, and the numbers of chromosomes with aberrations and aneuploidy at both dose levels.

In the context of developmental susceptibility to formaldehyde exposure, as noted above, the respiratory tract lining fluid (RTLF) protecting the lungs is often lower in glutathione levels than is the RTLF of adult lungs (Reise et al., 1997). This is especially true in premature infants who later develop chronic lung disease (Grigg et al., 1993). As glutathione is central to the lungs' antioxidant defenses, and is involved in the metabolism of inhaled formaldehyde, this relative deficiency may make the neonate's and infant's developing lungs more susceptible to toxic insult. It should be noted that ascorbate is also an important component of the lung's antioxidant defense, especially when glutathione levels are depressed (Jain et al., 1992). In healthy lungs, ascorbate helps to maintain glutathione levels. However, as is the case for glutathione, ascorbate levels in RTLF drop during the first week following birth (Vyas et al., 2001), potentially adding to the neonate's susceptibility to glutathione-reactive substances. Indeed, alterations in lung development following early life air toxicant exposure has been shown for environmental tobacco smoke (Wang and Pinkerton, 2007) and ozone (Plopper et al., 2007). Whether early life exposure to formaldehyde has similar effects on lung development remains to be demonstrated. However, there is concern that allergen exposure can modulate trophic interactions of conducting airway epithelial and interstitial wall components (Finkelstein and Johnston, 2004) and alter postnatal development of the airways (Plopper et al., 2007). This, coupled with the ability of formaldehyde to enhance the immune response to proteins/allergens with which it binds (Thrasher et al., 1987, 1990), may render developing lungs more susceptible to formaldehyde exposure. If evidence of such developmental effects associated with formaldehyde exposure becomes available, a re-evaluation of the REL for formaldehyde may be necessary.

8. Derivation of Reference Exposure Levels

8.1 Formaldehyde Acute Reference Exposure Level

Study	Kulle et al., 1987
Study population	19 nonasthmatic, nonsmoking humans
Exposure method	Whole body to 0.5-3.0 ppm
Exposure continuity	Single exposure per concentration
Exposure duration	3 hr
Critical effects	mild and moderate eye irritation
LOAEL	1 ppm
NOAEL	0.5 ppm
Benchmark concentration	0.44 ppm
Time-adjusted exposure	not applied
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	not applied
Subchronic uncertainty factor (UFs)	not applied

Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	10
Reference Exposure Level	**55 μg/m^3 (44 ppb)**

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document).

Kulle et al (1987) was chosen as the critical study for the determination of the acute REL as it used a sensitive endpoint, eye irritation. It featured human subjects showing significant ($p < 0.05$) responses with short-term exposures to a range of formaldehyde concentrations, and the data permitted the use of a benchmark concentration (BMC) approach. As described in the technical support document, OEHHA recommends the use of the BMC approach whenever the available data support it as the BMC method provides a more statistically sound estimate of the point of departure in the REL determination.

The proposed acute REL was based on a $BMCL_{05}$ for eye irritation, estimated using log-probit analysis (Crump, 1984). The $BMCL_{05}$ is defined as the 95% lower confidence limit of the concentration expected to produce a response rate of 5%. The resulting $BMCL_{05}$ from this analysis was 0.44 ppm (0.53 mg/m^3) formaldehyde. The endpoint of eye irritancy appears to be more a function of formaldehyde concentration rather than duration of exposure (Yang et al., 2001), so no time correction factor was applied. An uncertainty factor (UF_{H-k}) of 1 was used since sensory irritation is not expected to involve large toxicokinetic differences among individuals. Although the toxicological endpoint is eye irritation, the REL should protect against all possible adverse effects. The respiratory irritant effect, with documented potential to exacerbate asthma, is clearly an effect with the potential to differentially impact infants and children. In addition, the ability of formaldehyde to exacerbate the immune response to aeroallergens is of especial concern during development of the lungs. The toxicodynamic component of the intraspecies uncertainty factor UF_{H-d} is therefore assigned an increased value of 10 to account for potential asthma exacerbation. These considerations are applied equally to the acute, 8-hour and chronic REL.

As noted in Section 5.1, contact lens wearers appear to be at greater risk for ocular irritation with formaldehyde exposure. However, since contact lens users, and infants and children are generally mutually exclusive groups, it is expected that with the ten-fold toxicodynamic UF_{H-d} described above, the acute REL should be adequately protective of these individuals as well.

8.2 Formaldehyde 8-Hour Reference Exposure Level

Study	Wilhelmsson and Holmstrom, 1992
Study population	66 chemical plant workers
Exposure method	Discontinuous occupational exposure
Exposure continuity	8 hr/day, 5 days/week (assumed)
Exposure duration	10 years (average); range 1-36 years
Critical effects	Nasal obstruction and discomfort, lower airway discomfort, and eye irritation.
LOAEL	Mean 0.26 mg/m^3 (range 0.05 – 0.6 mg/m^3) (described as exposed group)
NOAEL	Mean of 0.09 mg/m^3 (described as control group of office workers)
Benchmark concentration	not derived
Time-adjusted exposure	0.09 mg/m^3 (time adjustment not applied)
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	1 (NOAEL observed)
Subchronic uncertainty factor (UFs)	not applied
Interspecies Uncertainty Factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	10
Reference Exposure Level	**9 μg/m^3 (7 ppb)**

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the Technical Support Document).

The 8-hour REL is based on the occupational study by Wilhelmsson and Holmstrom (1992). This study evaluated the effects of formaldehyde on the upper airways of adult human subjects exposed to a mean formaldehyde concentration of 0.26 mg/m^3 during the work day compared with a referent group exposed to 0.09 mg/m^3. The critical effects in this study included nasal obstruction and discomfort, lower airway discomfort, and eye irritation. A NOAEL and a LOAEL may be derived from these data but no other dose-response information was provided. This study included only adults, but there is evidence that children may be more susceptible to long term exposures to formaldehyde than are adults. Thus, in the absence of child-specific data, an intraspecies uncertainty factor of 10 for toxicodynamic variability and developmental susceptibility was applied.

For comparison, the 8-hour REL of 9 µg/m³ is similar to the value of 10 µg/m^3 based on increased pulmonary resistance in guinea pigs following an 8 hr exposure to 0.11 – 1.05 ppm formaldehyde (Swiecichowski et al., 1993). The NOAEL of 0.59 ppm in guinea pigs was adjusted to a Human Equivalent Concentration (HEC) of 0.49 ppm with a regional gas dose ratio (RGDR) of 0.826. Use of the HEC adjustment entails an interspecies uncertainty factor of 6, while an intraspecies uncertainty factor of 10 addresses toxicodynamic variability.

Study	Swiecichowski et al., 1993
Study population	25-35 adult male guinea pigs
Exposure method	Whole body exposure
Exposure continuity	
Exposure duration	8 hr
Critical effects	Increased specific pulmonary resistance
LOAEL	1.0 ppm
NOAEL	0.59 ppm
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	0.49 ppm (610 µg/m^3) (0.59 * RGDR 0.826 for pulmonary effects)
LOAEL uncertainty factor (UF_L)	1 (default: NOAEL observed)
Subchronic uncertainty factor (UFs)	not applied
Interspecies Uncertainty Factor	
Toxicokinetic (UF_{A-k})	6 (with HEC adjustment)
Toxicodynamic (UF_{A-d})	1 (with HEC adjustment)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF_{H-k})	1 (no systemic effect)
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	60
Reference Exposure Level	**10 µg/m³ (8 ppb)**

8.3 Formaldehyde Chronic Reference Exposure Level

Study	Wilhelmsson and Holmstrom, 1992 supported by Edling et al., 1988
Study population	66 human chemical plant workers
Exposure method	Discontinuous occupational exposure
Exposure continuity	8 hr/day, 5 days/week (assumed)
Exposure duration	10 years (average); range 1-36 years
Critical effects	Nasal obstruction and discomfort, lower airway discomfort.
LOAEL	Mean 0.26 mg/m^3 (range 0.05 – 0.6 mg/m3) (described as exposed group)
NOAEL	Mean of 0.09 mg/m^3 (described as control group of office workers)
Benchmark concentration	not derived
Time-adjusted exposure	0.09 mg/m^3 for NOAEL group
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	not applied
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (no systemic effects)
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	10
Reference Exposure Level	**9 µg/m^3 (7 ppb)**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

The study by Wilhelmsson and Holmstrom (1992) was selected for development of the chronic REL as it investigated long-term exposure to formaldehyde relatively free of other confounding exposures. From this study it was possible to determine both a NOAEL and a LOAEL. Since this study included only adults, a combined intraspecies uncertainty factor of 10 for toxicodynamic variability was applied to account for the possibly greater susceptibility of children with long term exposures to formaldehyde.

The susceptibility of young children was examined in a study by Rumchev et al. (2002) that compared children (mean age 25 mo) with a clinical diagnosis of asthma to children without this diagnosis. The LOAEL used (60 µg/m^3) represents the formaldehyde level at which the authors found a statistically elevated risk for asthma-related respiratory symptoms. For this comparison, the NOAEL was taken to be 30 µg/m^3, the lower end of the NOAEL range. Intraspecies uncertainty factors of 3.16 for potential toxicodynamic variability and 1 for toxicokinetic

differences give a cumulative uncertainty factor of 3.16 for an inhalation chronic REL of 10 μg/m^3 (8 ppb), similar to the chronic REL calculated from the critical study.

Study	Rumchev et al., 2002
Study population	88 asthmatic children (mean age 25 mo); 104 nonasthmatic controls (mean age 20 mo)
Exposure method	Ambient in home
Exposure continuity	Continuous assumed
Exposure duration	range 0.5-3 years
Critical effects	Parent-reported respiratory symptoms (cough, shortness of breath, wheeze, trouble breathing)
LOAEL	60 μg/m^3
NOAEL	30 μg/m^3 (lower limit of NOAEL range)
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	30 μg/m^3
LOAEL uncertainty factor (UF_L)	1
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (study performed in children)
Toxicodynamic (UF_{H-d})	√10 (inter-individual variation)
Cumulative uncertainty factor	√10
Reference Exposure Level	**10 μg/m^3 (8 ppb)**

The Rumchev study supports an association with exposure to formaldehyde and the observation of asthma symptoms (cough, shortness of breath, wheeze, trouble breathing) in children. However, it was not selected for REL development due to the difficulties in distinguishing asthma from other wheezing conditions in the clinical diagnoses in such a young population. There are additional uncertainties associated with the exposure continuity, and the possibility of observational and/or recall bias in the parental reports of respiratory symptoms characteristic of asthma.

For comparison with the chronic REL of 9 μg/m^3 (7 ppb) presented above, Table 8.3.1 below presents a summary of potential formaldehyde RELs based on chronic and subchronic animal studies originally presented in OEHHA (2000). The toxicological endpoint was nasal lesions, consisting principally of rhinitis, squamous metaplasia, and dyplasia of the respiratory epithelium.

The most striking observation is the similarity of potential RELs among the rat chronic studies (exposures $\geq$ 26 weeks) that contain a NOAEL. The range of RELs from these animal studies, 1.5 – 24.9 ppb, includes the proposed REL (7 ppb) based on a human study. Another related observation is that the NOAEL and LOAEL are similar among all the studies, regardless of exposure duration. The NOAEL and LOAEL are generally in the range of 1 - 4 ppm and 1 – 10 ppm, respectively, with the exception of the study by Kamata et al. (1997) that may be due to the

absence of a dose level between 2 and 0.3 ppm. It is also of interest that the studies of Rusch et al (1983) indicate that monkeys and rats are of about the same sensitivity. In addition, the results of the Rusch studies suggest that, at least for the endpoint of squamous metaplasia, formaldehyde concentration is more important than the total dose since these animals, receiving more continuous exposure, exhibited the same adverse effects seen in studies using more intermittent exposures.

ATSDR has estimated minimum risk levels (MRLs), defined as "an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure" (ATSDR, 1999). For formaldehyde inhalation exposures they describe as "acute" (≤ 14 days), the MRL is 40 ppb based on a LOAEL of 0.4 ppm from a study by Pazdrak et al. (1993), and a 9-fold uncertainty factor (3 for use of a LOAEL; 3 for intraspecies variability). This exposure period is much longer than the acute REL of one hour, but the acute REL represents possibly repeated exposures. The MRL for an "intermediate" exposure period of 15-364 days is 30 ppb based on a NOAEL of 0.98 ppm for clinical signs of nasopharyngeal irritation and lesions in the nasal epithelium in monkeys (Rusch et al., 1983). A chronic MRL (≥ 365 d) of 8 ppb was developed based on damage to nasal epithelium in chemical factory workers (Holmstrom et al., 1989). This number is similar to the chronic REL of 7 ppb reported here. The MRLs are more similar to the chronic RELs developed by OEHHA in that they assume continuous exposure over the specified time period rather than regular but periodic exposures, as assumed for the 8-hour RELs considered above. For 8-hr exposures, NIOSH (1988) suggested a TWA 8-hr REL of 16 ppb based on sensory irritation.

8.4 Formaldehyde as a Toxic Air Contaminant

Formaldehyde was identified by the ARB as a toxic air contaminant (TAC) in accordance with sections 39660-39662 of the California Health and Safety Code on March 12, 1992 (Title 17, California Code of Regulations, section 93001)(CCR, 2007). In view of the differential impacts on infants and children identified in Section 6.2, OEHHA recommends that formaldehyde be listed as a toxic air contaminant which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

Table 8.3.1. Summary of Chronic and Subchronic Formaldehyde Studies in Experimental Animals

Study	Animal	Duration	Exposure	LOAEL ppm	NOAEL ppm	Time adj	DAF	LOAEL UF	UFak	UFad	UFhk	UFhd	UFsc	Cum UF	REL ppb	REL µg/m3
Woutersen 89	rat	28 mo	6 h 5 d	9.8	1	0.179	0.148	1	1	3.16	1	10	1	30	4.9	6.1
Kerns 83	rat	24 mo	6 h 5 d	2	n/a	0.357	0.296	6	1	3.16	1	10	1	200	1.5	1.8
Monticello 96	rat	24 mo	6 h 5 d	6.01	2.05	0.366	0.304	1	1	3.16	1	10	1	30	10.1	12.6
Kamata 97	rat	24-28 mo	6 h 5 d	2	0.3	0.054	0.044	1	1	3.16	1	10	1	30	1.5	1.8
Appelman 88	rat	52 wk	6 h 5 d	9.4	1	0.179	0.148	1	1	3.16	1	10	1	30	4.9	6.1
Rusch 83	rat	26 wk	22 h 7d	2.95	0.98	0.898	0.746	1	1	3.16	1	10	1	30	24.9	30.8
Kimbell 97	rat	26 wk	6 h 5 d	6	2	0.357	0.296	1	1	3.16	1	10	1	30	9.9	12.3
Wilmer 89	rat	13 wk	8 h 5 d	4	2	0.238	0.198	1	1	3.16	1	10	1	30	6.6	8.2
Woutersen 87	rat	13 wk	6 h 5 d	9.7	1	0.179	0.148	1	1	3.16	1	10	1	30	4.9	6.1
Zwart 88	rat	13 wk	6 h 5 d	2.98	1.01	0.180	0.15	1	1	3.16	1	10	1	30	5.0	6.2
Kerns 83	mouse	24 mo	6 h 5 d	5.6	2	0.357	0.296	1	2	3.16	1	10	1	60	4.9	6.1
Maronpot 86	mouse	13 wk	6 h 5 d	10.1	4.08	0.729	0.605	1	2	3.16	1	10	1	60	10.1	12.5
Rusch 83	monkey	26 wk	22 h 7d	2.95	0.98	0.898	not used	1	2	2	1	10	1	40	22.5	27.8

9. References

Akbar-Khanzadeh F, Vaquerano MU, Akbar-Khanzadeh M and Bisesi MS (1994). Formaldehyde exposure, acute pulmonary response, and exposure control options in a gross anatomy laboratory. Am J Ind Med 26(1): 61-75.

Alarie Y (1981). Toxicological evaluation of airborne chemical irritants and allergens using respiratory reflex reactions. . Proceedings of the inhalation toxicology and technology symposium.Ann Arbor Sciences, Inc. 207-231. Kalamazoo, MI, October 23-24, 1980.

Alexandersson R and Hedenstierna G (1988). Respiratory hazards associated with exposure to formaldehyde and solvents in acid-curing paints. Arch Environ Health 43(3): 222-7.

Alexandersson R and Hedenstierna G (1989). Pulmonary function in wood workers exposed to formaldehyde: a prospective study. Arch Environ Health 44(1): 5-11.

Alexandersson R, Hedenstierna G and Kolmodin-Hedman B (1982). Exposure to formaldehyde: effects on pulmonary function. Arch Environ Health 37(5): 279-84.

Amdur MO (1960). The response of guinea pigs to inhalation of formaldehyde and formic acid alone and with a sodium chloride aerosol. Int J Air Pollut 3: 201-20.

Apfelbach R and Weiler E (1991). Sensitivity to odors in Wistar rats is reduced after low-level formaldehyde-gas exposure. Naturwissenschaften 78(5): 221-3.

Appelman LM, Woutersen RA, Zwart A, Falke HE and Feron VJ (1988). One-year inhalation toxicity study of formaldehyde in male rats with a damaged or undamaged nasal mucosa. J Appl Toxicol 8(2): 85-90.

ATSDR. (1999). *Toxicological profile for formaldehyde*. Atlanta, GA: Agency for Toxic Substances and Disease Registry

http://www.atsdr.cdc.gov/toxprofiles/tp111.pdf.

Boja JW, Nielsen JA, Foldvary E and Truitt EB, Jr. (1985). Acute low-level formaldehyde behavioural and neurochemical toxicity in the rat. Prog Neuropsychopharmacol Biol Psychiatry 9(5-6): 671-4.

Boysen M, Zadig E, Digernes V, Abeler V and Reith A (1990). Nasal mucosa in workers exposed to formaldehyde: a pilot study. Br J Ind Med 47(2): 116-121.

Broder I, Corey P, Brasher P, Lipa M and Cole P (1988). Comparison of health of occupants and characteristics of houses among control homes and homes insulated with urea formaldehyde foam. III. Health and house variables following remedial work. Environ Res 45(2): 179-203.

Burge PS, Harries MG, Lam WK, O'Brien IM and Patchett PA (1985). Occupational asthma due to formaldehyde. Thorax 40(4): 255-60.

CARB (2005a). Annual Statewide Toxics Summary - Formaldehyde. Sacramento, CA. http://www.arb.ca.gov/adam/toxics/statepages/hchostate.html.

CARB. (2005b). *The California Almanac of Emissions and Air Quality - 2005 Edition*. California Air Resources Board. http://www.arb.ca.gov/aqd/almanac/almanac05/almanac05.htm.

CARB. (2006). *The California Almanac of Emissions and Air Quality - 2006 Edition*. California Air Resources Board. http://www.arb.ca.gov/aqd/almanac/almanac06/almanac2006all.pdf.

CCR (2007). California Code of Regulations Section 93001 Hazardous Air Pollutants Identified as Toxic Air Contaminants. Sacramento, CA: California Office of Administrative Law. 8-20-07. http://ccr.oal.ca.gov/linkedslice/default.asp?SP=CCR-1000&Action=Welcome.

Cross CE, van der Vliet A, O'Neill CA, Louie S and Halliwell B (1994). Oxidants, antioxidants, and respiratory tract lining fluids. Environ Health Perspect 102 Suppl 10: 185-91.

Crump KS (1984). A new method for determining allowable daily intakes. Fundam Appl Toxicol 4(5): 854-71.

Dallas CE, Badeaux P, Theiss JC and Fairchild EJ (1989). The influence of inhaled formaldehyde on rat lung cytochrome P450. Environ Res 49(1): 50-9.

Edling C, Hellquist H and Odkvist L (1988). Occupational exposure to formaldehyde and histopathological changes in the nasal mucosa. Br J Ind Med 45(11): 761-5.

Feinman SE (1988). Formaldehyde sensitivity and toxicity. Boca Raton (FL): CRC Press Inc.

Finkelstein JN and Johnston CJ (2004). Enhanced sensitivity of the postnatal lung to environmental insults and oxidant stress. Pediatrics 113(4 Suppl): 1092-6.

Franklin P, Dingle P and Stick S (2000). Raised exhaled nitric oxide in healthy children is associated with domestic formaldehyde levels. Am J Respir Crit Care Med 161(5): 1757-9.

Franks SJ (2005). A mathematical model for the absorption and metabolism of formaldehyde vapour by humans. Toxicology and Applied Pharmacology 206(3): 309-320.

Frigas E, Filley WV and Reed CE (1984). Bronchial challenge with formaldehyde gas: lack of bronchoconstriction in 13 patients suspected of having formaldehyde-induced asthma. Mayo Clin Proc 59(5): 295-9.

Garrett MH, Hooper MA, Hooper BM, Rayment PR and Abramson MJ (1999). Increased risk of allergy in children due to formaldehyde exposure in homes. Allergy 54(4): 330-7.

Gaston B, Sears S, Woods J, Hunt J, Ponaman M, McMahon T and Stamler JS (1998). Bronchodilator S-nitrosothiol deficiency in asthmatic respiratory failure. Lancet 351(9112): 1317-9.

Gorski P and Krakowiak A (1991). Formaldehyde--induced bronchial asthma--does it really exist? [Abstract]. Pol J Occup Med Environ Health 4(4): 317-20.

Gorski P, Tarkowski M, Krakowiak A and Kiec-Swierczynska M (1992). Neutrophil chemiluminescence following exposure to formaldehyde in healthy subjects and in patients with contact dermatitis. Allergol Immunopathol (Madr) 20(1): 20-3.

Grammer LC, Harris KE, Shaughnessy MA, Sparks P, Ayars GH, Altman LC and Patterson R (1990). Clinical and immunologic evaluation of 37 workers exposed to gaseous formaldehyde. J Allergy Clin Immunol 86(2): 177-81.

Green DJ, Sauder LR, Kulle TJ and Bascom R (1987). Acute response to 3.0 ppm formaldehyde in exercising healthy nonsmokers and asthmatics. Am Rev Respir Dis 135(6): 1261-6.

Grigg J, Barber A and Silverman M (1993). Bronchoalveolar lavage fluid glutathione in intubated premature infants. Arch Dis Child 69(1 Spec No): 49-51.

Harving H, Korsgaard J, Pedersen OF, Molhave L and Dahl R (1990). Pulmonary function and bronchial reactivity in asthmatics during low-level formaldehyde exposure. Lung 168(1): 15-21.

Hendrick DJ and Lane DJ (1977). Occupational formalin asthma. Br J Ind Med 34(1): 11-8.

Holmstrom M and Wilhelmsson B (1988). Respiratory symptoms and pathophysiological effects of occupational exposure to formaldehyde and wood dust. Scand J Work Environ Health 14(5): 306-11.

Holmstrom M, Wilhelmsson B and Hellquist H (1989). Histological changes in the nasal mucosa in rats after long-term exposure to formaldehyde and wood dust. Acta Otolaryngol 108(3-4): 274-83.

Horton AW, Tye R and Stemmer KL (1963). Experimental carcinogenesis of the lung. Inhalation of gaseous formaldehyde or an aerosol of coal tar by C3H mice. J Natl Cancer Inst 30: 31-43.

Horvath EP, Jr., Anderson H, Jr., Pierce WE, Hanrahan L and Wendlick JD (1988). Effects of formaldehyde on the mucous membranes and lungs. A study of an industrial population. JAMA 259(5): 701-7.

IARC. (2006). Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol; Summary of data reported and evaluation. International Agency for Research on Cancer. Lyon, France

Jain A, Martensson J, Mehta T, Krauss AN, Auld PA and Meister A (1992). Ascorbic acid prevents oxidative stress in glutathione-deficient mice: effects on lung type 2 cell lamellar bodies, lung surfactant, and skeletal muscle. Proc Natl Acad Sci U S A 89(11): 5093-7.

Jensen DE, Belka GK and Du Bois GC (1998). S-Nitrosoglutathione is a substrate for rat alcohol dehydrogenase class III isoenzyme. Biochem J 331 (Pt 2): 659-68.

Kamata E, Nakadate M, Uchida O, Ogawa Y, Suzuki S, Kaneko T, Saito M and Kurokawa Y (1997). Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats. J Toxicol Sci 22(3): 239-54.

Kerfoot EJ and Mooney TF (1975). Formaldehyde and paraformaldehyde study in funeral homes. Am Ind Hyg Assoc J 36(7): 533-7.

Kerns WD, Pavkov KL, Donofrio DJ, Gralla EJ and Swenberg JA (1983). Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Res 43(9): 4382-92.

Kilburn KH, Warshaw R and Thornton JC (1989). Pulmonary function in histology technicians compared with women from Michigan: effects of chronic low dose formaldehyde on a national sample of women. Br J Ind Med 46(7): 468-72.

Kim CW, Song JS, Ahn YS, Park SH, Park JW, Noh JH and Hong CS (2001). Occupational asthma due to formaldehyde. Yonsei Medical Journal 42(4): 440-445.

Kimbell JS, Gross EA, Richardson RB, Conolly RB and Morgan KT (1997). Correlation of regional formaldehyde flux predictions with the distribution of formaldehyde-induced squamous metaplasia in F344 rat nasal passages. Mutat Res 380(1-2): 143-54.

Kitaeva LV, Kitaev EM and Pimenova MN (1990). [The cytopathic and cytogenetic sequelae of chronic inhalational exposure to formaldehyde on female germ cells and bone marrow cells in rats]. Tsitologiia 32(12): 1212-6.

Krakowiak A, Gorski P, Pazdrak K, Ruta U, Wantke F, Focke M, Hemmer W, Tschabitscher M, Gann M, Tappler P, Gotz M and Jarisch R (1998). Airway response to formaldehyde inhalation in asthmatic subjects with suspected respiratory formaldehyde sensitization. Formaldehyde and phenol exposure during an anatomy dissection course: a possible source of IgE-mediated sensitization? Am J Ind Med 33(3): 274-81.

Kriebel D, Myers D, Cheng M, Woskie S and Cocanour B (2001). Short-term effects of formaldehyde on peak expiratory flow and irritant symptoms. Arch Environ Health 56(1): 11-8.

Krzyzanowski M, Quackenboss JJ and Lebowitz MD (1990). Chronic respiratory effects of indoor formaldehyde exposure. Environ Res 52(2): 117-25.

Kulle TJ, Sauder LR, Hebel JR, Green DJ and Chatham MD (1987). Formaldehyde dose-response in healthy nonsmokers. Japca 37(8): 919-24.

Lang I, Bruckner T and Triebig G (2008). Formaldehyde and chemosensory irritation in humans: A controlled human exposure study. Regul Toxicol Pharmacol 50(1): 23-36.

Levine RJ, Andjelkovich DA and Shaw LK (1984). The mortality of Ontario undertakers and a review of formaldehyde-related mortality studies. J Occup Med 26(10): 740-6.

Liley HG, White RT, Benson BJ and Ballard PL (1988). Glucocorticoids both stimulate and inhibit production of pulmonary surfactant protein A in fetal human lung. Proc Natl Acad Sci U S A 85(23): 9096-100.

Liu KS, Huang FY, Hayward SB, Wesolowski J and Sexton K (1991). Irritant effects of formaldehyde exposure in mobile homes. Environ Health Perspect 94: 91-4.

Malaka T and Kodama AM (1990). Respiratory health of plywood workers occupationally exposed to formaldehyde. Arch Environ Health 45(5): 288-94.

Malek FA, Moritz KU and Fanghanel J (2004). Effects of a single inhalative exposure to formaldehyde on the open field behavior of mice. Int J Hyg Environ Health 207(2): 151-8.

Mannino DM, Homa DM, Pertowski CA, Ashizawa A, Nixon LL, Johnson CA, Ball LB, Jack E and Kang DS (1998). Surveillance for asthma--United States, 1960-1995. MMWR CDC Surveill Summ 47(1): 1-27.

Maronpot RR, Miller RA, Clarke WJ, Westerberg RB, Decker JR and Moss OR (1986). Toxicity of formaldehyde vapor in B6C3F1 mice exposed for 13 weeks. Toxicology 41(3): 253-66.

Martin WJ (1990). A teratology study of inhaled formaldehyde in the rat. Reprod Toxicol 4(3): 237-9.

Monteiro-Riviere NA and Popp JA (1986). Ultrastructural evaluation of acute nasal toxicity in the rat respiratory epithelium in response to formaldehyde gas. Fundam Appl Toxicol 6(2): 251-62.

Monticello TM, Swenberg JA, Gross EA, Leininger JR, Kimbell JS, Seilkop S, Starr TB, Gibson JE and Morgan KT (1996). Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. Cancer Res 56(5): 1012-22.

Nagornyi PA, Sudakova Zh A and Shchablenko SM (1979). [General toxic and allergic action of formaldehyde]. Gig Tr Prof Zabol(1): 27-30.

Nielsen GD, Hougaard KS, Larsen ST, Hammer M, Wolkoff P, Clausen PA, Wilkins CK and Alarie Y (1999). Acute airway effects of formaldehyde and ozone in BALB/c mice. Hum Exp Toxicol 18(6): 400-9.

NIOSH. (1988). *Current Intelligence Bulletin 50: Carcinogenic effects of exposure to diesel exhaust*. Centers for Disease Control.

Nordman H, Keskinen H and Tuppurainen M (1985). Formaldehyde asthma--rare or overlooked? J Allergy Clin Immunol 75(1 Pt 1): 91-9.

OEHHA. (2000). *The Air Toxics Hot Spots Program Risk Assessment Guidelines Part III: Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels*. OEHHA. http://www.oehha.ca.gov/air/chronic_rels/pdf/relsP32k.pdf.

Olsen JH and Dossing M (1982). Formaldehyde induced symptoms in day care centers. Am Ind Hyg Assoc J 43(5): 366-70.

Pazdrak K, Gorski P, Krakowiak A and Ruta U (1993). Changes in nasal lavage fluid due to formaldehyde inhalation. Int Arch Occup Environ Health 64(7): 515-9.

Plopper CG, Smiley-Jewell SM, Miller LA, Fanucchi MV, Evans MJ, Buckpitt AR, Avdalovic M, Gershwin LJ, Joad JP, Kajekar R, Larson S, Pinkerton KE, Van Winkle LS, Schelegle ES, Pieczarka EM, Wu R and Hyde DM (2007). Asthma/allergic airways disease: does postnatal exposure to environmental toxicants promote airway pathobiology? Toxicol Pathol 35(1): 97-110.

Porter JA (1975). Letter: Acute respiratory distress following formalin inhalation. Lancet 2(7935): 603-4.

Pross HF, Day JH, Clark RH and Lees RE (1987). Immunologic studies of subjects with asthma exposed to formaldehyde and urea-formaldehyde foam insulation (UFFI) off products. J Allergy Clin Immunol 79(5): 797-810.

Que LG, Liu L, Yan Y, Whitehead GS, Gavett SH, Schwartz DA and Stamler JS (2005). Protection from experimental asthma by an endogenous bronchodilator. Science 308(5728): 1618-21.

Reise JA, Taylor GW, Fardy CH and Silverman M (1997). Glutathione and neonatal lung disease. Clin Chim Acta 265(1): 113-9.

Reynaert NL, Ckless K, Wouters EF, van der Vliet A and Janssen-Heininger YM (2005). Nitric oxide and redox signaling in allergic airway inflammation. Antioxid Redox Signal 7(1-2): 129-43.

Riedel F, Hasenauer E, Barth PJ, Koziorowski A and Rieger CH (1996). Formaldehyde exposure enhances inhalative allergic sensitization in the guinea pig. Allergy 51(2): 94-9.

Ritchie IM and Lehnen RG (1987). Formaldehyde-related health complaints of residents living in mobile and conventional homes. Am J Public Health 77(3): 323-8.

Rumchev KB, Spickett JT, Bulsara MK, Phillips MR and Stick SM (2002). Domestic exposure to formaldehyde significantly increases the risk of asthma in young children. Eur Respir J 20(2): 403-8.

Rusch GM, Clary JJ, Rinehart WE and Bolte HF (1983). A 26-week inhalation toxicity study with formaldehyde in the monkey, rat, and hamster. Toxicol Appl Pharmacol 68(3): 329-43.

Saillenfait AM, Bonnet P and de Ceaurriz J (1989). The effects of maternally inhaled formaldehyde on embryonal and foetal development in rats. Food Chem Toxicol 27(8): 545-8.

Salem H and Cullumbine H (1960). Inhalation toxicities of some aldehydes. Toxicol Appl Pharmacol 2: 183-7.

Sari DK, Kuwahara S, Tsukamoto Y, Hori H, Kunugita N, Arashidani K, Fujimaki H and Sasaki F (2004). Effect of prolonged exposure to low concentrations of formaldehyde on the corticotropin releasing hormone neurons in the hypothalamus and adrenocorticotropic hormone cells in the pituitary gland in female mice. Brain Research 1013(1): 107-116.

Sauder LR, Chatham MD, Green DJ and Kulle TJ (1986). Acute pulmonary response to formaldehyde exposure in healthy nonsmokers. J Occup Med 28(6): 420-4.

Sauder LR, Green DJ, Chatham MD and Kulle TJ (1987). Acute pulmonary response of asthmatics to 3.0 ppm formaldehyde. Toxicol Ind Health 3(4): 569-78.

Schachter EN, Witek TJ, Jr., Brody DJ, Tosun T, Beck GJ and Leaderer BP (1987). A study of respiratory effects from exposure to 2.0 ppm formaldehyde in occupationally exposed workers. Environ Res 44(2): 188-205.

Schachter EN, Witek TJ, Jr., Tosun T, Leaderer BP and Beck GJ (1986). A study of respiratory effects from exposure to 2 ppm formaldehyde in healthy subjects. Arch Environ Health 41(4): 229-39.

Sheppard D, Eschenbacher WL and Epstein J (1984). Lack of bronchomotor response to up to 3 ppm formaldehyde in subjects with asthma. Environ Res 35(1): 133-9.

Skog E (1950). A toxicological investigation of lower aliphatic aldehydes. Acta Pharmacol 6: 299-318.

Solomons K and Cochrane JW (1984). Formaldehyde toxicity. Part I. Occupational exposure and a report of 5 cases. S Afr Med J 66(3): 101-2.

Sorg BA, Bailie TM, Tschirgi ML, Li N and Wu W-R (2001b). Exposure to repeated low-level formaldehyde in rats increases basal corticosterone levels and enhances the corticosterone response to subsequent formaldehyde. Brain Research 898(2): 314-320.

Sorg BA, Tschirgi ML, Swindell S, Chen L and Fang J (2001a). Repeated formaldehyde effects in an animal model for multiple chemical sensitivity. Ann NY Acad Sci 933(Role of Neural Plasticity in Chemical Intolerance): 57-67.

Srivastava AK, Gupta BN, Bihari V, Gaur JS, Mathur N and Awasthi VK (1992). Clinical studies of employees in a sheet-forming process at a paper mill. Vet Hum Toxicol 34(6): 525-7.

Staab CA, Alander J, Brandt M, Lengqvist J, Morgenstern R, Grafstrom RC and Hoog JO (2008). Reduction of S-nitrosoglutathione by alcohol dehydrogenase 3 is facilitated by substrate alcohols via direct cofactor recycling and leads to GSH-controlled formation of glutathione transferase inhibitors. Biochem J 413(3): 493-504.

Sugiura H and Ichinose M (2008). Oxidative and nitrative stress in bronchial asthma. Antioxid Redox Signal 10(4): 785-97.

Swiecichowski AL, Long KJ, Miller ML and Leikauf GD (1993). Formaldehyde-induced airway hyperreactivity in vivo and ex vivo in guinea pigs. Environ Res 61(2): 185-99.

Tanaka K, Nishiyama K, Yaginuma H, Sasaki A, Maeda T, Kaneko SY, Onami T and Tanaka M (2003). [Formaldehyde exposure levels and exposure control measures during an anatomy dissecting course]. Kaibogaku Zasshi 78(2): 43-51.

Thompson CM and Grafstrom RC (2008). Mechanistic considerations for formaldehyde-induced bronchoconstriction involving S-nitrosoglutathione reductase. J Toxicol Environ Health A 71(3): 244-8.

Thrasher JD, Broughton A and Madison R (1990). Immune activation and autoantibodies in humans with long-term inhalation exposure to formaldehyde. Arch Environ Health 45(4): 217-23.

Thrasher JD, Wojdani A, Cheung G and Heuser G (1987). Evidence for formaldehyde antibodies and altered cellular immunity in subjects exposed to formaldehyde in mobile homes. Arch Environ Health 42(6): 347-50.

Uba G, Pachorek D, Bernstein J, Garabrant DH, Balmes JR, Wright WE and Amar RB (1989). Prospective study of respiratory effects of formaldehyde among healthy and asthmatic medical students. Am J Ind Med 15(1): 91-101.

Vyas JR, Currie A, Dunster C, Kelly FJ and Kotecha S (2001). Ascorbate acid concentration in airways lining fluid from infants who develop chronic lung disease of prematurity. Eur J Pediatr 160(3): 177-84.

Wallenstein G, Rebohle E, Bergmann I, Voigt U and Schneider WD (1978). [Occupational diseases of the respiratory system due to chemical substances with potential allergen effects]. Dtsch Gesundheitsw 33(24): 1119-23.

Wang L and Pinkerton KE (2007). Air pollutant effects on fetal and early postnatal development. Birth Defects Res C Embryo Today 81(3): 144-54.

Wantke F, Demmer CM, Tappler P, Gotz M and Jarisch R (1996). Exposure to gaseous formaldehyde induces IgE-mediated sensitization to formaldehyde in school-children. Clin Exp Allergy 26(3): 276-80.

Wantke F, Focke M, Hemmer W, Bracun R, Wolf-Abdolvahab S, Gotz M, Jarisch R, Gotz M, Tschabitscher M, Gann M and Tappler P (2000). Exposure to formaldehyde and phenol during an anatomy dissecting course: sensitizing potency of formaldehyde in medical students. Allergy 55(1): 84-87.

Weber-Tschopp A, Fischer T and Grandjean E (1977). [Irritating effects of formaldehyde on man (author's transl)]. Int Arch Occup Environ Health 39(4): 207-18.

Wilhelmsson B and Holmstrom M (1992). Possible mechanisms of formaldehyde-induced discomfort in the upper airways. Scand J Work Environ Health 18(6): 403-7.

Wilmer JW, Woutersen RA, Appelman LM, Leeman WR and Feron VJ (1989). Subchronic (13-week) inhalation toxicity study of formaldehyde in male rats: 8-hour intermittent versus 8-hour continuous exposures. Toxicol Lett 47(3): 287-93.

Witek TJ, Jr., Schachter EN, Tosun T, Beck GJ and Leaderer BP (1987). An evaluation of respiratory effects following exposure to 2.0 ppm formaldehyde in asthmatics: lung function, symptoms, and airway reactivity. Arch Environ Health 42(4): 230-7.

Woutersen RA, van Garderen-Hoetmer A, Bruijntjes JP, Zwart A and Feron VJ (1989). Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. J Appl Toxicol 9(1): 39-46.

Wu H, Romieu I, Sienra-Monge JJ, Estela Del Rio-Navarro B, Anderson DM, Jenchura CA, Li H, Ramirez-Aguilar M, Del Carmen Lara-Sanchez I and London SJ (2007). Genetic variation in S-nitrosoglutathione reductase (GSNOR) and childhood asthma. J Allergy Clin Immunol 120(2): 322-8.

Yang X, Zhang YP, Chen D, Chen WG and Wang R (2001). Eye irritation caused by formaldehyde as an indoor air pollution--a controlled human exposure experiment. Biomed Environ Sci 14(3): 229-36.

Yi C, Ke K, Xiaohua L and Xu Y (2007). Up-regulation of GSNO reductase in mice lungs by formaldehyde inhalation. Bioinform Biomed Engineering 6-8: 294-297.

Zaman K, Hanigan MH, Smith A, Vaughan J, Macdonald T, Jones DR, Hunt JF and Gaston B (2006). Endogenous S-nitrosoglutathione modifies 5-lipoxygenase expression in airway epithelial cells. Am J Respir Cell Mol Biol 34(4): 387-93.

Zwart A, Woutersen RA, Wilmer JW, Spit BJ and Feron VJ (1988). Cytotoxic and adaptive effects in rat nasal epithelium after 3-day and 13-week exposure to low concentrations of formaldehyde vapour. Toxicology 51(1): 87-99.

Manganese and Compounds Reference Exposure Levels

1. Summary

Acute inhalation of high levels of manganese results in a nonspecific pulmonary edema, while chronic manganese inhalation leads to a characteristic neurotoxicity known as manganism with strong similarities to Parkinson's disease. Manganism is characterized by motor deficits (dystonia, altered gait, fine tremor, generalized rigidity) and may include psychiatric disturbances. At low manganese levels and in the absence of frank manganism, subtle deficits in cognitive and neurobehavioral functions have been reported in both adults and children. Neurodevelopmental deficits have been associated with early life exposure to excessive manganese and include impaired intellectual performance and behavioral disinhibition. The studies described in this document include those published through the Spring of 2008. The RELs below are applicable to all respirable inorganic manganese compounds.

1.1 Manganese Acute REL

An acute REL for manganese was not developed at this time.

1.2 Manganese 8-Hour REL

Reference Exposure Level	**0.17 µg/m^3**
Critical effect(s)	Impairment of neurobehavioral function in humans
Hazard index target	Nervous system

1.3 Manganese Chronic REL

Reference Exposure Level	**0.09 µg/m^3**
Critical effect(s)	Impairment of neurobehavioral function in humans
Hazard index target(s)	Nervous system

2. Physical and Chemical Properties

Table 2.1 Manganese and Manganese Species*

Molecular Formula	*Synonyms*	*Molecular Weight*	*CAS Reg. No.*
Mn	elemental manganese; colloidal manganese; cutaval	54.94 g/mol	7439-96-5
MnO	manganese oxide; manganese monoxide; manganosite	70.94 g/mol	1344-43-0
MnO_2	manganese dioxide; black manganese oxide	86.94 g/mol	1313-13-9
Mn_3O_4	manganese tetroxide; trimanganese tetraoxide; manganomanganic oxide	228.82 g/mol	1317-35-7
$MnCl_2$	manganese chloride; manganese dichloride; manganous chloride	125.84 g/mol	7773-01-5
$MnSO_4$	manganese sulfate	151.00 g/mol	7787-85-7
$KMnO_4$	potassium permanganate	158.03 g/mol	7724-64-7

Description	Lustrous, gray-pink metal (Mn); green (MnO), black (MnO_2) or pink ($MnCl_2$, $MnSO_4$), purple ($KMnO_4$) crystals; brownish-black powder (Mn_3O_4)
Molecular formula	see Table 2.1
Molecular weight	see Table 2.1
Density (in g/cm^3)	7.21-7.4 (Mn – depending on allotropic form); 5.43-5.46 (MnO); 4.88 (Mn_3O_4); 2.977 @ 25°C ($MnCl_2$)
Boiling point	2095°C (Mn); not available (MnO); not available (Mn_3O_4); 1190°C ($MnCl_2$); 850°C ($MnSO_4$)
Melting point	1246°C (Mn); 1839°C (MnO); 1567°C (Mn_3O_4); 650°C ($MnCl_2$) (CRC, 2005); 700°C ($MnSO_4$)
Vapor pressure	1 torr @ 1292°C (Mn); non-volatile at room temperature (Mn_3O_4); not available (MnO; $MnCl_2$)
Solubility	Sol. in dil. acids and aq. solns. of Na- or K-bicarbonate (Mn); sol. in NH_4Cl, insol. in H_2O (MnO); insol. in H_2O, HNO_3, or cold H_2SO_4 (MnO_2 (Merck, 1976)); insol. in H_2O, sol. in HCl (Mn_3O_4); 72.3 g/100 ml H_2O @ 25°C ($MnCl_2$); sol in 1 part H_2O ($MnSO_4$); 72.3 g/100 ml H_2O ($KMnO_4$)
Conversion factor	Not applicable (dusts or powders)

3. Occurrence and Major Uses

Metallic manganese is used in the manufacturing of steel, carbon steel, stainless steel, cast iron, and superalloys to increase hardness, stiffness, and strength (HSDB, 2006). Manganese chloride is used in dyeing, disinfecting, batteries, and as a paint drier and dietary supplement. Manganese oxide (MnO) is used in textile printing, ceramics, paints, colored glass, fertilizers, and as food additives and supplements. Manganese dioxide is used in batteries and may also be generated from the welding of manganese alloys. Use of manganese-containing welding rods is a major source of occupational exposure to welders. Manganese tetroxide may be generated in situations where other oxides of manganese are heated in air (NIOSH, 2005). Manganese is also released into the air during the erosion of manganese-containing rock and alloys. Relatively high levels of manganese have been measured in subways (428 ng/m^3 vs 9.7 ng/m^3 ambient), presumably from the frictional erosion of manganese-containing steel (Crump, 2000). As methylcyclopentadienyl manganese tricarbonyl (MMT), manganese has found use as an octane enhancer in some unleaded gasolines and is released during fuel combustion as manganese sulfate, phosphate, and oxides. Manganese exposure may also be significant among farm workers using the fungicide Maneb (manganese ethylene-bis-dithiocarbamate).

Manganese is present in ambient air as particles, often associated with other metals or organic material. The size of these particles depends on their source, history and contents. For example, Singh et al. (2002) compared the metal contents and size distributions of particles at two sites in the Los Angeles Basin, Downey, which is in the vicinity of downtown Los Angeles and Riverside, 70 km east of Los Angeles. In Downey, 7% of the manganese was in ultrafine particles ≤ 0.1 µm, 38% was in fine particles of 0.35-1.0 µm, and less than 20% in the coarse (PM 2.5-10 µm) fraction. By comparison, in Riverside, less than 2% of the manganese was in ultrafine PM (≤ 0.1 µm) 8% in the 0.35-1.0 range, while nearly 80% was in the 2.5-10 µm fraction (Table 3.1).

TABLE 3.1 PARTICLE SIZE AND MANGANESE DISTRIBUTION

Particle size (µm)	Downey	Riverside
2.5-10	18%	77%
1-2.5	21%	12%
0.35-1	38%	8%
0.1-0.35	16%	1.5%
≤ 0.1	7%	1.3%

In contrast to Riverside, manganese measured in other urban settings tends to be mainly in the respirable fine fraction. In urban aerosols in Seville, Spain, manganese was found predominantly in fine particles < 0.61 µm (44%), with smaller amounts in coarse particles (> 10 µm, 17.8%; 4.9-10 µm, 18.3%) (Espinoza et al., 2001). A bimodal distribution of sizes was also found for manganese-containing aerosols in Tihany, Hungary. The bulk of the manganese was found bound to organic matter and silicates in particles of approximately 0.118 µm, with smaller amounts in the 1.4-2.8 µm range (Hlavay et al., 1998). In cities in which the gasoline contains MMT, manganese is also predominantly in the fine (PM 2.5) fraction (Pellizzari et al., 2001).

Since combustion is associated with the production of ultrafines, more research on airborne ultrafine manganese particles in areas where MMT is present in the gasoline is warranted

The 2004 annual statewide emissions of manganese reported in the most recent California Toxics Inventory (CARB, 2005a) were estimated to be 1,055 tons. For 2002, the mean statewide ambient level was 31.5 ng/m^3.

4. Metabolism / Toxicokinetics

Environmental manganese can enter the body primarily by oral and inhalation routes. Dermal absorption of manganese is insignificant through intact skin; however, broken skin would obviously allow more access to manganese (e.g., potassium permanganate) and other poorly dermally absorbed compounds. Parenteral exposures have occurred through parenteral feeding and more recently i.v. drug abuse, leading to human disease. Manganese is an essential element normally absorbed from the intestinal tract as part of the diet. It is estimated that 2 to 5% of ingested manganese is retained in the adult body (Andersen et al., 1999). Retention can be up to 41% in breast-fed infants, and 20% in formula-fed infants (Dorner et al., 1989). Manganese absorption is increased (along with iron absorption) when there is a deficiency of iron in the diet (Davis et al., 1992). Ascorbic acid, calcium and phosphorus also affect manganese utilization (ibid).

As part of the normal manganese homeostatic mechanism, high levels of dietary manganese diminish absorption from the intestinal tract. Manganese appears to be absorbed from the gut largely in the divalent form, with approximately 80% of absorbed manganese subsequently bound in plasma to $ß_1$-globulin and albumin (Foradori et al., 1967). These manganese-protein complexes are efficiently removed from the blood by the liver and returned to the gut in bile for elimination, thus establishing an entero-hepatic circuit for manganese. In the blood, unbound manganese may be converted by ceruloplasmin to the trivalent cation which is then bound by transferrin. Transferrin-manganese complexes are much less efficiently removed by the liver and thus survive first pass elimination to circulate throughout the body (Gibbons et al., 1976). In the brain, transferrin receptors in the capillary beds may mediate uptake in regions with efferents to the nucleus accumbens and the caudate putamen. Other mechanisms also appear to contribute to brain uptake of manganese including a divalent metal transporter (DMT-1), and a less well-defined non-saturable mechanism. From these sites, manganese is thought to move by neuronal transport to the pallidum, thalamic nuclei, and substantia nigra, areas involved with motor control and movement (Aschner et al., 2005). While at normal plasma levels, manganese enters the brain mainly across the capillary epithelium, at elevated levels of manganese in the blood, transport across the choroid plexus becomes more prominent (Aschner, 2000).

The mechanisms mentioned above are thought to apply generally to the transport of manganese across the blood brain barrier of adults. However, in the fetus and neonate, the blood brain barrier is characterized as having greater permeability to many substances, including manganese, and a different distribution of molecular transporters (Erikson et al., 2007b). In mice following a single parenteral administration of $MnCl_2$ on postnatal days 7, 14, or 42, maximum retention of manganese in the brain occurred 24 hours after exposure and was 3.5%, 2.5%, and 0.3%, respectively, of the administered dose. For manganese administered on day 0, the maximum brain concentration (2.9%) occurred 43 days later, suggesting a lack of perinatal homeostatic

control (Valois and Webster, 1989). The approximately ten-fold higher brain levels following dosing on days 0-14 compared with day 42 indicate a more rapid and extensive uptake of manganese from the blood in neonates compared with adults. The drop in maximum brain levels between days 14 and 42 is thought to reflect attainment of adult blood brain barrier function on day 21.

Manganese may be introduced directly into the blood during parenteral feeding or during injection of illicit designer drugs contaminated with permanganate. As with the inhalation route described below, parenteral administration of manganese avoids first pass clearance of manganese by the liver, and may result in high exposure of all organs to manganese.

Manganese exposure via the pulmonary route leads to more rapid absorption with higher efficiency, and with greater transfer to the brain compared with other routes (Drown et al., 1986; Roels et al., 1997). In experiments in 3-month old rats, Roels et al. (1997) used intratracheal instillation as a surrogate for inhalation for comparison with the oral route (gavage). Intratracheal instillation of $MnCl_2$ (1.22 mg/kg, once weekly for four weeks) raised the steady state manganese levels 68% in blood, 205% in the striatum, 48% in the cortex, and 27% in the cerebellum compared to controls. By gavage, a much higher dose of $MnCl_2$ (24.3 mg/kg) was required to achieve the same blood levels (68%). However, by this route, manganese levels in the striatum and cerebellum were not affected, and levels in the cortex were raised by only 22% (Table 4.1). In animals given a single intratracheal dose of $MnCl_2$ (1.22 mg/kg bw), blood manganese levels peaked within 30 min at 7,050 ng/100 ml. This was followed by a gradual decline but blood levels remained elevated over controls for at least 24 hours. By comparison, the single oral administration of 24.3 mg $MnCl_2$/kg bw resulted in a five-fold lower peak blood level of 1,660 ng/100 ml after one hour, followed by a return to control levels in 12 hours. Thus, compared to ingestion, inhalation of a relatively water soluble form of manganese leads to a rapid increase in blood levels that remain higher for longer, and results in higher brain manganese levels.

TABLE 4.1 INCREASE IN TISSUE MANGANESE BY ROUTE AND CHEMICAL FORM

	Increase in Tissue Manganese (%)			
Chemical Form and Route	Blood	Striatum	Cortex	Cerebellum
$MnCl_2$ Intratracheal (1.2 mg/kg)	68	205	48	27
$MnCl_2$ Gavage (24.3 mg/kg)	68	0	22	0
MnO_2 Intratracheal (1.2 mg/kg)	41	48	34	31
MnO_2 Gavage (24.3 mg/kg)	0	0	0	0

Using the same exposure protocol with the less soluble MnO_2, intratracheal instillation raised manganese levels 41% in blood, 48% in striatum, 31% in cerebellum, and 34% in cortex. By contrast, neither blood nor brain levels were increased following oral exposure (Table 4.1). As with $MnCl_2$, Mn blood levels following intratracheal MnO_2 reached a higher peak value (1,760 ng Mn/100 ml; 200% increase) than that achieved after gavage (900 ng/100 ml; 27% increase). Blood levels rose more slowly than with $MnCl_2$, starting at 48–72 hr after intratracheal instillation and peaking at 168 hr. By gavage, blood levels rose gradually to peak at 144 hr

(Roels et al., 1997). In these studies, the solubility of the manganese complexes influenced the rate of absorption by either route, but in both cases inhalation resulted in substantially higher blood and brain levels.

In a further demonstration of the dependence of tissue distribution on oxidation state and route of exposure, Reaney et al. (2006) exposed 8-month old rats to 0, 2, or 6 mg/kg Mn(III)-pyrophosphate or $Mn(II)Cl_2$ intraperitoneally (i.p.) for five weeks. Significantly higher blood manganese levels were seen with Mn(III) vs equimolar Mn(II). A dose-dependent increase in brain manganese was observed, with Mn(III) producing levels that were 25% higher than following Mn(II). This may be related to the higher blood levels of manganese achieved with Mn(III) vs Mn(II) via the i.p. route. Examination of the striatum, globus pallidus, thalamus, and cerebral cortex by PIXE (particle induced x-ray emission; an x-ray fluorescence technique) revealed no differences in the distribution of manganese across these brain regions. There were, however, differences among regions in response to the concentration and oxidation state of the manganese. In the globus pallidus, the highest cumulative dose (90 mg/kg) of both forms of manganese increased GABA levels compared to controls (15-30%, $p = 0.037$). By contrast, dopamine levels in globus pallidus at this dose were increased by 60% with Mn(III), but decreased by 40% with Mn(II). The mechanism behind this differential effect is not clear but suggests that manganese oxidation states are important in manganese toxicity.

Drown and colleagues studied the distribution of soluble $^{54}MnCl_2$ and insoluble $^{54}Mn_3O_4$ after instillation into the adult rat lung (Drown et al., 1986). Initially the soluble form of manganese distributed more rapidly from the lung to the peripheral tissues than did the insoluble form. After two weeks the rates of distribution of the two forms became almost equal. Manganese (^{54}Mn) reached higher concentrations in the liver, kidney, and gastrointestinal tissues, but persisted longer in the heart, brain, and bone. The manganese was eliminated mainly in bile with very little elimination in urine.

The influence of solubility on tissue distribution was examined with aerodynamically similar aerosols of three manganese compounds of differing solubilities ($MnPO_4$, $MnSO_4$, and Mn_3O_4) in rats following inhalation (6 hr/day, 14 days) at 0, 0.03, 0.3, and 3.0 mg/m^3. At comparable dose levels, animals exposed to $MnSO_4$ had lower lung manganese levels than those exposed to either Mn_3O_4 (Dorman et al., 2001) or $MnPO_4$ (Vitarella et al., 2000), suggesting more rapid pulmonary clearance of the most soluble form. Consistent with this observation, after exposure to 3 mg/m^3, manganese levels in the olfactory bulb and striatum were highest with $MnSO_4$, followed by Mn_3O_4, then $MnPO_4$. As observed by Drown et al. (1986) and Roels et al. (1997) for intratracheal instillation, the more soluble forms of manganese accumulate in the brain more quickly following inhalation than do the less soluble compounds (Normandin et al., 2004).

For humans with occupational and/or environmental exposures, the main route of exposure is via inhalation. In both cases the manganese is usually in the form of particulates of various sizes. Manganese deposited in the lung can be absorbed directly into the blood stream, or can migrate (by mucociliary transport) into the upper respiratory tract and then be swallowed for possible absorption in the GI tract. In experimental animals, inhaled manganese may be transported via olfactory nerves directly to the brain following absorption from nasal passages (Brenneman et al., 2000; Dorman et al., 2002a; Elder et al., 2006; Dorman et al., 2006a). Neither pulmonary nor gastrointestinal absorption is required for this route of exposure, and the blood-brain barrier is

bypassed. Evidence for absorption of particulate manganese oxide from the nose and transport to the brain was provided by Elder et al. (2006) in rats. Manganese concentrations in the olfactory bulb increased 3.5-fold following 12 days of intranasal instillation of ultrafine manganese oxide particles (3-8 nm) in both nares. With occlusion of the right nostril and instillation in the left naris, manganese accumulated almost exclusively in the left olfactory bulb. In this experimental paradigm, instillation of either the soluble manganese chloride or the insoluble manganese oxide particles (solubilization rate 1-1.5% per day) in the patent naris resulted in comparable levels of manganese in the ipsilateral side of the olfactory bulb. This, in conjunction with the observation that an increase in manganese in the olfactory bulb was detectable within 30 minutes of the instillation, suggests that particulate rather than dissolved manganese was the form transported to the brain. While instillation is not inhalation, this study does indicate absorption of manganese ultrafine particles occurs across the nasal epithelium with direct transport to the brain.

The deposition and uptake of manganese from the upper and lower airways is also influenced by particle size. Rats with nose-only exposure to MnO_2 aerosols of 1.3 and 18 µm mass median aerodynamic diameters (MMAD) showed higher levels of manganese in the lungs and olfactory bulbs following 15 days of inhalation exposure to the smaller (1.3 µm) versus larger (18 µm) particles (Fechter et al., 2002). Thus, while there was greater deposition of large particle manganese in the nasal passages compared with small particles, uptake from the nose was more efficient with the smaller particles. In addition, for the smaller particles, the lungs were a larger reservoir for more continuous systemic uptake of inhaled manganese. Thus the evaluations of potential toxicity from inhaled manganese must consider not only the chemical form of manganese, but also the particle size as important determinants of the toxicokinetics. This may be particularly relevant to combustion products of MMT in which particle size is of 0.2-0.4 µm (Ter Haar et al., 1975). The inhalation toxicity of manganese-containing particles in this size range has received less attention than the larger fine and coarse particles.

More evidence for direct nose to brain transport in primates was provided by magnetic resonance imaging (MRI) studies in adult rhesus monkeys exposed by inhalation to manganese sulfate for 6 hours/day, 5 days/week for 13 weeks (Dorman et al., 2006a). Increases in signal intensity on T1-weighted images of various brain regions were well correlated with manganese levels measured upon necropsy. Increases in mean pallidal manganese concentrations of approximately 1.7-, 2.7-, and 6-fold over air-exposed controls were seen following exposure to $MnSO_4$ at 0.19, 0.97, and 4.55 mg/m^3, respectively. The particles at these three concentrations had MMAD of 1.73, 1.12, and 2.12 µm, respectively (1.04, 1.07, 1.12 µm geometric mean diameter (GMD), respectively). As expected, much higher increases in manganese concentrations were observed in olfactory epithelium, bulb, tract, and cortex. Lower but statistically significant ($p < 0.05$) increases were also observed in the putamen, white matter, and cerebellum. This study provided no evidence for translocation of manganese from the olfactory bulb to other brain regions, consistent with uptake from the blood as the source of manganese in the globus pallidus. However, the resolution of the MRI used in this study did not allow visualization of individual nerve tracts to rule out direct transfer of manganese between brain regions. Nevertheless, this study provides evidence for axonal transport from the nasal epithelium at least as far as the olfactory bulb in primates.

The major route of excretion of manganese is via bile, although a lesser amount is excreted via urine (Davis et al., 1993). That the liver maintains homeostasis of manganese can be seen by the fact that patients with cirrhosis of the liver accumulate abnormally high levels of manganese in their brains, especially in the globus pallidus (Rose et al., 1999). Similarly, rats that have a liver bypass also show high levels of manganese in the brain, especially in the globus pallidus (Rose et al., 1999).

Neonatal humans do not excrete manganese for the first two to three weeks of life. The intestinal barrier to manganese absorption is also immature in premature and neonatal infants (Cawte, 1985).

The toxicokinetics of manganese may also influence and be influenced by other metals. There is evidence that manganese uptake from the intestinal tract (Mena, 1974; Erikson et al., 2002), lungs (Brain et al., 2006), and nose (Thompson et al., 2007), is enhanced by iron deficiency. Rats rendered anemic by periodic bleeding absorbed significantly higher amounts of manganese (Brain et al., 2006). In each of these studies, brain levels of manganese were increased by iron deficiency. Based on data from NHANES III, the prevalence of iron deficiency among infants 1-2 years of age was 9%, and 9-11% among adolescent girls and women of child-bearing age. For comparison, the prevalence among teenage boys and men less than 50 years of age was 1%. Among older children and adults over 50 years of age, the prevalence of iron deficiency did not exceed 7% (Looker et al., 1997). Inasmuch as iron deficiency is a widespread condition that disproportionately affects the young (Beard et al., 2001), children represent a more susceptible population.

Manganese may exist in eleven different valence states (-3 to +7) and may participate in a variety of oxidation-reduction reactions as a pro- or anti-oxidant. In biological systems, while the divalent (Mn^{2+}) and trivalent (Mn^{3+}) forms are most abundant, the trivalent form predominates in many tissues and appears to be responsible for manganese's pro-oxidant properties, possibly by its participation in Fenton-type reactions (HaMai et al., 2001). In vitro studies attempting to emulate conditions in the brain have shown that Mn^{3+}, but not Mn^{2+} (both as pyrophosphate), oxidizes dopamine, DOPA (a dopamine precursor), norepinephrine, and epinephrine to quinones and other products, with reduction of Mn^{3+} to $Mn(OH)_2$ (Archibald and Tyree, 1987). Polymerization of these catecholamine-derived quinones to form neuromelanin, from which the substantia nigra derives its name, is an O_2^--generating, auto-oxidative process that in turn enhances oxidation of Mn^{2+} to Mn^{3+}, thus increasing cellular oxidative stress.

The role of oxidative stress in manganese toxicity has been inferred in part from changes in cellular markers of oxidative stress upon exposure to high levels of manganese. As a result of participation in reactions with reactive oxygen species (ROS), levels of GSH in manganese-exposed cells decrease. This decrease may also reflect binding of GSH by manganese. Where metals are involved in the generation of ROS, metallothionein levels typically rise. Other markers, such as glutamine synthetase and tyrosine hydroxylase, are used due to their sensitivity to cellular oxidation states. In the hypothalamus of rats, subchronic inhalation exposure to $MnSO_4$ (0.03, 0.3, 3.0 mg/m^3) has been associated with a decrease in GSH and an increase in metallothionein mRNA, while the olfactory bulb experienced an increase in glutamine synthetase (Dobson et al., 2003). Similarly, subchronic inhalation of $MnSO_4$ (0.06, 0.3, 1.5 mg/m^3) by rhesus monkeys resulted in decreased tyrosine hydroxylase, glutamate transporter-1,

glutamate/aspartate transporter, and glutamine synthetase (Erikson et al., 2007a). These changes reflect exposure to oxidative stress that impairs neurotransmitter synthesis, while increased metallothionein mRNA in all the brain regions examined (caudate, cerebellum, frontal cortex, globus pallidus, olfactory cortex, putamen) is a cellular response to ameliorate the effects of reactive oxygen species. Many of these effects were significantly different from controls starting at the lowest manganese concentration tested (0.06 mg/m^3). While these studies of manganese have focused on the role of metal-induced oxidative stress, the ability of manganese to bind to sulfhydryl groups, exemplified by GSH, suggests the possibility that manganese may also bind protein sulfhydryls. Indeed, the depletion of both protein- bound and non-protein sulfhydryls by manganese has been demonstrated in rat brains (Shukla and Chandra, 1977), and the possibility that such interactions alter the structure/function of key proteins has been theorized to represent an important mode of manganese toxicity (Martin, 1986).

The ability of manganese to readily bind thiol groups can enhance cellular susceptibility to oxidative stress by directly depleting GSH levels. However, manganese may also lower GSH levels indirectly by enhancing the autooxidation of cysteine, the rate limiting precursor to glutathione (Wang and Cynader, 2001). In addition to making it unavailable for glutathione synthesis, the autooxidation of cysteine generates free radicals that are cytotoxic. Manganese III may oxidize cellular thiols, such as GSH and cysteine, to thiyl radicals (Wariishi et al., 1989). These may in turn participate in the oxidation of critical cellular components or signalling molecules as exemplified by the oxidation of dopamine by the cysteinyl thiyl radical (Shen and Dryhurst, 1998).

The effects of manganese on the markers of oxidative stress described above show an age and sex dependency. Juvenile (8 weeks) male and female rats, and senescent males (18 mo) breathed atmospheres containing $MnPO_4$ at 0.099 or $MnSO_4$ at 0.01, 0.098, or 0.478 mg Mn/m^3 (1.85, 1.92, 2.03 µm MMAD) for 6 hr/day, 5 day/wk for 13 weeks (Erikson et al., 2004). In young males, but not females or senescent males, there was an increase in glutamine synthetase levels in the hippocampus, but a decrease in the hypothalamus with both forms of manganese that was significant ($p < 0.05$) with exposure to $MnPO_4$. With exposure to the medium dose of $MnSO_4$, female and old, but not young, male rats showed significant decreases in glutamine synthetase levels in the hippocampus. Total GSH levels significantly decreased in the olfactory bulb of young males, but increased in females. In the striatum, GSH levels were significantly decreased in females and old males at all doses of $MnSO_4$, but were largely unchanged in young males. This is interesting in light of the observation by Dorman et al. (2004) that neither old age nor gender influenced delivery of manganese to the striatum. The decrease of GSH in the striatum in aged rats may be a result of the age-related loss of dopaminergic neurons, while the effect in females is suggested to be related to differences in levels of sex hormones between males and females. These data indicate that toxicokinetic and toxicodynamic characterizations of manganese must take age and gender into account.

That the chemical form and oxidation state of manganese is critical to its toxicokinetics is evident from the foregoing. No less critical to the toxicokinetics is the presence of manganese on or as nanoparticles versus free Mn_3O_4 in solution. The ability of manganese to cause oxidative stress in cultured human lung epithelial cells was assessed by measurement of reactive oxygen species (ROS) (Limbach et al., 2007). Nanoparticles (20-75 nm) of pure manganese, or silica doped with 0.5 and 1.6 wt % manganese, were suspended in culture medium at 30 ppm for

comparison with the more soluble Mn_3O_4 at comparable concentrations. Compared with pure silica nanoparticles, particles doped with as little as 1.6 wt% manganese increased ROS in cultured cells by 2,500% while the free Mn_3O_4 increased ROS by only 400%. For comparison, in similar experiments Co_3O_4 particles were less than half as potent. In cell-free culture medium, ROS production was not different between the particles and the dissolved salt. This suggests that it is the dissolved metal, not the particles per se, that is responsible for the ROS generation, and that the nanoparticles function to facilitate manganese uptake by the cell.

5. Acute Toxicity of Manganese

Acute inhalation exposure to high levels of manganese as its oxides is associated with pulmonary edema and impaired function (Shiotsuka, 1984). The very small body of literature on acute toxicity includes two animal experiments involving acute exposures by inhalation. One is a two-hour exposure of 200 female CD-1 mice to manganese oxide (Mn_3O_4) aerosols (Adkins et al., 1980) that resulted in a NOAEL of 2.9 mg/m^3 based on respiratory effects (edema). The other is a 24 hr exposure of guinea pigs to 22 mg/m^3 MnO_2 (Bergstrom, 1977) that examined the effects of manganese exposure on pulmonary leukocytes, macrophages, and the clearance of bacteria from the lungs. However, since no dose response in lung wet:dry weight ratios was observed, no LOAEL was reported in the Adkins study, and the Bergstrom study employed a single exposure level. The accumulation of manganese in brain structures following acute inhalation exposure (Newland et al., 1987; Brenneman et al., 2000; Dorman et al., 2002a), and following intranasal instillation (Gianutsos et al., 1997) has been described; however, the toxicological consequences of these exposures were not reported. Neurobehavioral effects have been observed in mice following acute subcutaneous injections (Dodd et al., 2005), and in rats after a single oral administration of manganese (Shukakidze et al., 2003), but it is not clear how these routes of exposure compare to inhalation. No studies of acute manganese inhalation were located that demonstrated a dose-response or evaluated other toxicological endpoints.

6. Chronic Toxicity of Manganese

6.1 Chronic Toxicity to Adult Humans

Exposure of humans to manganese by inhalation leads to a suite of neurological effects called "manganism" (Lucchini et al., 1999). Frank manganism is a progressive disease that involves symptoms similar to those of Parkinson's disease. Manganism is characterized by altered gait, fine tremor and occasionally psychiatric disturbances. The psychiatric disturbances are seldom seen in Parkinson's disease, although dementia sometimes occurs late in this disease. Despite their similarities, the symptoms of manganism and Parkinson's disease differ somewhat (Barbeau, 1984; Calne et al., 1994). Both manganism and Parkinson's disease involve generalized bradykinesia and widespread rigidity. However, tremor is less frequent and dystonia more frequent in manganism. Manganism is also distinguished by a propensity to fall backward, failure to achieve a sustained therapeutic response to levodopa, and failure to detect a reduction in fluorodopa uptake by positron emission tomography (Calne et al., 1994). In Parkinsonism, the damage appears to be confined to the substantia nigra, whereas in manganism the damage is more widespread, involving other parts of the basal ganglia (Huang et al., 1998).

Manganese accumulates in certain brain structures, especially the extrapyramidal system. Structures rich in dopaminergic neurons show a heightened sensitivity to manganese toxicity. Within these tissues, manganese is found preferentially in mitochondria where it disrupts oxidative phosphorylation and mitochondrial function (Gavin et al., 1999). Cytochrome c, released from damaged mitochondria, leads to apoptosis and loss of neurons (Malecki, 2001). Trivalent manganese can promote the formation of reactive oxygen species (HaMai et al., 2001) that can cause oxidative stress, which in turn has been shown to lead to apoptosis of neurons in the rat brain (Dobson et al., 2003). While individuals exposed to massive amounts of manganese show frank neurological symptoms as in the Groote Eylandt studies (Kilburn, 1987) and the industrial workers studies, individuals exposed to lesser amounts of manganese show more subtle neurological deficits in neurobehavioral tasks (Wennberg et al., 1992; Lucchini et al., 1999).

Adverse effects may occur at manganese exposure levels that are too low to cause frank manganism. Lucchini and his co-workers studied a group of 61 Italian ferroalloy workers who had been exposed to low levels of manganese dust by inhalation (Lucchini et al., 1999). These workers did not exhibit the frank signs of manganism, but they did exhibit subtler neurofunctional changes. The workers were exposed to a "current overall value" of 54 µg Mn per m^3 air at the time of the study, and an estimated average of 70.83 µg Mn dust/m^3 per year over an average 15.17 years of exposure. Earlier exposures were higher. In order to obtain a measure of cumulative exposure the investigators calculated a "cumulative exposure index" (CEI) for each worker based on their exposure history in the factory. For the purposes of analysis, workers were separated into three CEI groups of low CEI (< 500 µg/m^3 x yrs), mid CEI (500-1800 µg/m^3 x yrs), and high CEI (> 1800 µg/m^3 x yrs). The CEIs correlated positively with blood manganese levels. The workers were subjected to symptom questionnaires and neurobehavioral and neurophysiological testing for the purpose of finding whether neurological effects correlated with cumulative exposure. In multiple regression analyses, positive correlations were found between the log of the CEI and the following tests of the Swedish Performance Evaluations System: finger tapping in the dominant ($R = 0.32$, $p = 0.01$) and non-dominant ($R = 0.32$, $p = 0.01$) hands, Symbol Digit ($R = 0.33$, $p = 0.01$) and Digit Span ($R = 0.44$, $p = 0.004$). The moderate but significant correlation coefficients reported in this study suggest that manganese is an important contributor to these effects but likely not the only one. In addition, these results demonstrate that subtle neurological changes are taking place in workers exposed to relatively low levels of manganese in the absence of frank manganism. To identify safe exposure levels, the authors took the geometric mean of the mid CEI group (1113 µg/m^3 x yrs) and divided this by the geometric mean exposure time (11.51 yrs) to derive a value of 96.71 µg/m^3. When the low CEI group is used as the control group, this value represents the LOAEL for the observed neurobehavioral symptoms.

A battery of neurofunctional tests was also employed by Mergler et al. (1994) to document early nervous system dysfunction among workers with long-term (mean 16.7 yr) manganese exposure in a ferromanganese and silicomanganese alloy plant. Subjects (n = 115) were matched by age, educational level, and number of children to workers in the same geographical region but without exposure to metals or other neurotoxicants in the workplace. The test batteries assessed motor function (range, speed, stability, grip strength, manual dexterity, graphomotor) and sensory function (visual acuity, chromatic discrimination, contrast sensitivity, olfactory and vibrotactile threshold). A third battery assessed speech initiation and regulation, attention,

concentration and memory, cognitive flexibility, and affect. Environmental manganese levels were measured with personal monitors for total dust and manganese content (0.014 – 11.48 mg Mn/m^3), while stationary monitors measured manganese in respirable (0.001 – 1.273 mg/m^3) and nonrespirable dust. Manganese measured in blood was higher among manganese workers (1.03 vs 0.68 µg/100 ml, $p < 0.0001$), while urine levels were not significantly different.

On the tests of motor functions, the performance of manganese-exposed workers was significantly worse than controls ($p < 0.001$), with the greatest differences associated with tests requiring rapid, alternating, coordinated movements. While in the context of speech initiation and regulation there were no overall differences between groups, the manganese-exposed workers took significantly longer on the second trial of the digit naming test ($p = 0.05$) and made more errors ($p < 0.001$). Cognitive flexibility was also worse with manganese exposure ($p < 0.002$). Attention, concentration, and memory functions were similar between groups. In the comparison of mood states, manganese workers displayed significantly more tension ($p < 0.01$), anger ($p = 0.01$), fatigue ($p < 0.001$), and confusion ($p = 0.01$) than controls. The cross-sectional nature of this study precludes assigning symptoms of manganese toxicity to specific environmental levels of manganese since levels in the plant varied widely both spatially and likely historically. However, there was a strong association between blood levels of manganese and subtle neurobehavioral deficits.

Whether the neurobehavioral effects associated with occupational exposure to manganese are permanent or transitory has been the subject of several follow-up studies of occupational cohorts. Occupational exposure at the ferroalloy plant featured in the study above by Mergler et al. (1994) ceased with its closure in 1990. Fourteen years later, 69 of the original workers and 68 referents were re-examined with many of the same assessment tools used in the 1994 study (Bouchard et al., 2007a). After controlling for age, education, alcohol consumption, and smoking, manganese workers performed significantly worse on tests of motor function (Luria Motor Scale) than did referents in both the initial ($p < 0.001$) and follow-up ($p < 0.05$) evaluations, although the differences at follow-up were not as striking. The motor deficits that persisted between the initial and follow-up studies included slowing of simple and alternate movements, and poorer quality of form drawing. Deficits in hand steadiness observed in the initial study remained but were less pronounced at follow-up. These deficits were significantly associated with increasing levels of cumulative manganese exposure ($p < 0.05$). Although in the initial study, several tests of cognitive function showed significant deficits among the exposed workers, these differences were no longer evident at follow-up. In general, measures of mood states showed improvement over time. However, compared to referents, manganese workers tended to report more feelings of anger and hostility in both the initial and follow-up studies ($p < 0.1$). Feelings of confusion and bewilderment, while not different between groups in the initial study, were significantly more pronounced among manganese workers ($p < 0.05$) at follow-up and significantly associated with cumulative exposure ($p < 0.01$). In a neuropsychiatric profile, former manganese workers were significantly ($p < 0.05$) more likely to experience feelings of anger and depression than the referents (Bouchard et al., 2007b). In addition to the effects on the nervous system, in the present study former manganese workers had a ten-fold increase in risk of respiratory problems. These two studies suggest that with cessation of manganese exposure, there is improvement in some neurological functions, but deficits in others remain.

While studies of the effects of manganese have tended to emphasize occupational exposures, a similar constellation of neurobehavioral effects has been found in a community study from which those with occupational exposures were excluded. Mergler et al. (1999) assessed nervous system functions in 273 individuals (151 women, 122 men) randomly selected from those living in proximity to a former manganese production plant in Southwest Quebec. A battery of tests similar to those of the occupational study above (Mergler et al., 1994) was used to profile nervous system function in relation to blood manganese levels. Motor skills and coordination, learning and recall, visual perception and speed, verbal naming, and cognitive flexibility were assessed. Blood manganese levels ranged from 2.5 to 15.9 µg/l, with results stratified by blood levels (low < 7.5 µg/l vs high >7.5 µg/l), age and sex. Elevated blood manganese (> 7.5 µg/l) was associated with poorer upper limb coordination ($p = 0.04$) and deficits in learning and recall, which was stronger in men ($p = 0.002$) than in women ($p = 0.04$). These deficits were more pronounced in older subjects with elevated blood manganese. The neurobehavioral effects reported here were observed at blood manganese levels lower than those of the occupational study above (0.75 µg/ 100 ml vs 1.03 µg/100 ml). The authors thus suggest that "manganese neurotoxicity can be viewed as a continuum of dysfunction, with early, subtle changes at lower exposure levels, progressing to more severe neurological disorders at the high exposure levels that have been observed in mining, industry and agriculture."

Male workers (n = 92, plus 101 matched controls) in an alkaline battery plant in Belgium exposed to manganese dioxide dust were the subjects of a cross-sectional epidemiological study (Roels et al., 1992). Total manganese concentrations and manganese dust were measured in the workers' breathing zones with personal samplers. Lifetime integrated respirable dust levels (LIRD) ranged from 0.04 to 4.43 mg Mn/m^3 * year, with a geometric mean of 0.793 mg Mn/m^3 * year. The average age of control and exposed groups was 30 years with a mean manganese exposure time of 5.3 years (0.2 to 17.7 years) for the latter group. In exposed workers, the geometric mean levels of blood and urine manganese (corrected for creatinine) were significantly higher ($p < 0.001$) than in controls. The subjects were also evaluated for neurobehavioral function, lung function, and hematological parameters. There were no significant differences in respiratory symptoms between those exposed and controls, and hematological parameters were in the normal range for both groups. In neurobehavioral tests, significant decrements in performance were found in exposed workers on tests for visual reaction time ($p < 0.001$), five measures of eye-hand coordination ($p < 0.005$), and in two of three tests of hand tremor ($p < 0.03$). The data for individuals in this study were used in a BMD analysis to calculate the 8-hr and chronic RELs.

In 1999, Roels et al. (1999) published a follow-up study of the cohort in the Roels et al. (1992) study described above. During the course of the present study, covering the years 1988-1995, the cohort dropped from 92 to 34 workers. Three neurobehavioral assessments were made. Eye-hand coordination was tested yearly with an orthokinesimeter. Starting in 1991, yearly assessments were also made of visual reaction time, and hand steadiness with a hole tremormeter. Respirable manganese dust exposure was measured in a manner similar to that of the 1992 study using personal air monitors. Three levels of exposure (low, medium, and high), with average exposures from 1987-1992 of 400, 600, and 2,000 µg Mn/m^3, respectively, were compared with unexposed controls in a nearby chemical plant. After 1992, there was a substantial decline in manganese levels with the mean manganese levels dropping by the end of the study to 119, 181, and 744 $\mu g/m^3$ for the low, medium and high groups, respectively. In the

low exposure group, the test of eye-hand coordination showed improvement with the decreasing manganese levels, and results were normal by the end of the study, while the effects in the higher exposure groups persisted. The time courses of the hand steadiness and visual reaction time tests showed no improvement and suggested irreversible impairment. Similarly, in neurobehavioral assessments of workers who had ceased manganese exposure, eye-hand coordination significantly improved, but deficits in hand steadiness and visual reaction time remained. These studies suggest that some of the neurological deficits improve when manganese exposure decreases, while others may be permanent.

Another follow-up among workers in the same plant covered by Roels et al., 1992 and 1999 was conducted by Crump and Rousseau (1999) for the years 1985-1996. This study covered 213 workers including 114 of the 140 originally tested by Roels. In this study the metric for manganese exposure was blood and urine levels, neither of which was associated with memory or eye-hand coordination tests. There were, however, marginally significant associations between manganese levels and poorer hand steadiness ($p = 0.05$). As in the Roels et al. (1999) follow-up, some neurobehavioral deficits improved with time and lower manganese levels; others appeared to be more permanent.

Welding in confined spaces represents a setting in which significant occupational inhalation exposure to manganese may occur. In an evaluation of 43 welders working on the San Francisco Bay Bridge, Bowler et al. (2007) documented decrements on neurological, neurophysiological, and pulmonary tests associated with exposure to a time-weighted average manganese dust level of 0.11-0.46 mg/m^3 for an average of 16.5 months. Manganese blood levels exceeded 10 µg/l in 43% of the workers. Multiple regression analyses against blood manganese and/or the individual's cumulative exposure index (CEI) revealed significant inverse dose-effect relationships with IQ ($p \leq 0.05$), executive function ($p \leq 0.03$), sustaining concentration and sequencing ($p \leq 0.04$), verbal learning ($p \leq 0.01$), working memory ($p \leq 0.04$), and immediate memory ($p \leq 0.02$) after adjustment for demographics and years of welding before working on the Bay Bridge. Spirometric measurements, taken at three time points, indicated declining lung function with manganese exposure. The first time point was after working on the bridge for an average of 1.5 months, the second after 10.8 months, and the third after 20.9 months. Between the first and third time points, measures of lung function decreased: 7% for FEV_1, 2% for FVC, and 21,2% for the FEV_1:FVC ratio ($p < 0.05$). In tests of mood and affect, the levels of clinical depression and anxiety among welders were greater than two standard deviations above the normative mean. Neuropsychological tests of parameters characteristic of parkinsonism found that tremor was present 39-90% of the time on three different tests, postural sway was increased in about half of the welders, and motor dexterity and speed were impaired 52-95% of the time. Additional symptoms reported by the welders that showed significant negative correlations with the CEI included sexual function ($p < 0.05$), fatigue ($p < 0.05$), depression ($p < 0.01$), and headache ($p < 0.05$). Compared to test norms, olfaction was impaired in 88% of the welders. This study suffers from the absence of an unexposed control group for comparison. However, all welders were prescreened during the hiring process to ensure good health and fitness. Blood levels of copper, iron, and lead were also measured and considered to be in the normal range. This, coupled with the significant correlations between both blood and air manganese levels and the physiological and neurological decrements, strongly implicates manganese as a causative agent.

That an association between exposure to welding fumes and symptoms of neurotoxicity may be due to manganese was corroborated by magnetic resonance imaging (MRI) detection of characteristic bilateral hyperintense T1-weighted signals in the globus pallidus of eight welders referred for neurological assessment (Josephs et al., 2005). Among the six cases with multiple MRI follow-up scans, the intensity of the MRI signal among the four for whom manganese exposure was discontinued either remained the same (1 case) or faded (3 cases), indicating a loss of pallidal manganese. In the remaining two with continued exposure, the signal remained the same or increased in intensity. All cases presented multiple symptoms characteristic of manganism including postural tremors, reduced arm swing, ataxia, altered gait, multifocal myoclonus, and cognitive impairment. In addition, several cases reported irritability, memory loss, headaches, slurred speech, and reduced sexual drive. All cases had elevated or high normal serum manganese levels. This constellation of symptoms in association with manganese exposure and characteristic MRI images suggests a role for welding fumes in the development of manganism. Substantially similar symptomologies have been reported elsewhere in case studies of welders (Sadek et al., 2003). However, it is important to note that welding fumes are a mixture of metals, many of which are also neurotoxic and may contribute to the reported neurological symptoms.

The neurotoxic effects of exposure to welding fumes may be accompanied by pulmonary damage. Clara cells lining the airways normally secrete Clara cell protein (CC16) that has anti-inflammatory properties. Pulmonary damage that includes the Clara cells results in decreased recovery of CC16 in bronchoalveolar lavage fluid, as well as an increase in serum levels of CC16. The former effect is presumably due to reduced production by the affected Clara cells, while the latter is attributed to damage to the bronchoalveolar/blood barrier (Hermans et al., 1999). In ship welders, measures of serum CC16 levels, blood, urine and air manganese levels, pulmonary function (vital capacity), and subclinical neurological effects (EEG and visual evoked potential (VEP) were compared with unexposed controls (Halatek et al., 2005; 2008). During examinations that assessed both subjective and objective neurological status, 66% of the 59 workers reported subjective central nervous system symptoms, while 29% had abnormal VEP results and 41% had abnormal EEGs. Among welders showing neurological symptoms, blood manganese was significantly elevated (12.2 vs 6.1 µg/l, $p < 0.05$) while vital capacity was significantly depressed (84.5%, $p < 0.05$) (Halatek et al., 2008). Multiple linear regression analysis revealed strong partial correlations between abnormal VEP and EEG, and both blood manganese (0.72, $p = 0.03$) and an index of cumulative manganese exposure (0.66, $p = 0.01$) (Halatek et al., 2005). Levels of CC16 were significantly correlated (0.82, $p = 0.015$) with abnormal VEP, EEG and CNS symptoms. The CC16 levels were significantly lower (9.6 µg/l, $p < 0.05$) among the younger welders who had fewer years of exposure (3 years) but higher blood manganese levels (13.7 µg/l), abnormal VEP and EEG results, and depressed vital capacity (83%). The authors suggest that the elevated CC16 levels indicate that welding fume exposure compromises pulmonary function, including that of the bronchoalveolar barrier. This in turn facilitates manganese access to the blood and brain, with the attendant subclinical neuropathological changes.

As mentioned in the study above, in addition to neurotoxicity, manganese inhalation may lead to symptoms of pulmonary toxicity. Indeed, the incidence of respiratory disease is higher among manganese-exposed workers than those not exposed (Boojar and Goodarzi, 2002). In a case report, Wittczak et al. (2008) present the study of a 42-year-old non-smoking welder with

suspected occupational asthma. At admission, the patient presented with a recurrent nonproductive cough, and dyspnea with wheezing that usually occurred after 30-60 minutes of welding. Compared with non-work days, on the days the patient was exposed to welding fumes, he exhibited a greater than 20% variability in his peak expiratory flow rate (PEFR). Histamine challenge revealed significant bronchial hyperreactivity (PC_{20} = 0.5 mg/ml). In contrast to a placebo inhalation challenge (1% KCl solution), five minutes following a challenge with 0.1% MnCl solution, dyspnea occurred and forced expiratory volume (FEV1) dropped 45% . At one hour, FEV1 was 55% below resting levels, and only recovered to 35% below resting levels by 24 hours. At 4 and 24 hours post-exposure, changes in the proportions of eosinophils (8% and 10% resp.) and basophils (1% and 3%, resp.) were observed in induced sputum. None of these effects was observed in non-exposed controls after similar challenges. This constellation of symptoms and sensitivity to manganese challenge supports a role for manganese in occupational asthma.

Another occupational study of lower exposures was done in Sweden (Wennberg et al., 1992). In this study workers had been exposed for a year or more to manganese dust at mean concentrations of 0.18 mg/m^3 at one smelter, and 0.41 mg/m^3 at another. They were compared to workers at similar industrial plants without manganese exposure via a suite of neurological tests, including electroencephalogram, brainstem auditory evoked potential, event related auditory evoked potential, and diadochokinesometry (a test of the subject's ability to rotate a handle rapidly). Of these tests, the only one that produced significantly different results in the exposed subjects was the diadochokinesometry. The manganese-exposed workers were unable to rotate the handle as quickly as the control workers. This is interpreted as evidence of a "preclinical" effect of low-level manganese exposure.

A major study of non-industrial human exposures is the study of the natives of Groote Eylandt, a large island off the coast of Australia. The inhabitants of this island are Australian Aborigines. The island is so rich in manganese that the environment has been described as a "manganese ecology" (Kilburn, 1987). The inhabitants are exposed by virtually all routes of exposure, but especially by ingestion of food and water high in manganese. Kilburn studied the natives of Groote Eylandt and compared them to a control group of Australian Aborigines living in another part of Australia. This paper does not quantitate the manganese exposures or body levels of manganese in the study population, and it would be difficult to quantitate exposures in this complex environmental situation. Kilburn reports certain congenital abnormalities, such as deformations of the foot (talipes equinovarus), closed anus (imperforate anus), and anorectal malformations, and neurobehavioral problems, including progressive muscle wasting (amyotrophy) and failure of muscle coordination (ataxia), that apparently occur with greater frequency in the islanders than in the control groups, but these could also be due to genetic factors present in this small population. Indeed all of the problems were seen in just two pedigrees. A likely interpretation would be that the adverse health effects observed reflect gene-environment interactions.

Exposure to excess manganese may occur via the parenteral route, especially in individuals receiving total parenteral nutrition (TPN). Unfortunately, patients receiving TPN are often those with liver damage and/or gastrointestinal disorders, both of which compromise the hepatobiliary circuit by which the body regulates retention of dietary manganese. Manganese intoxication in these cases typically manifests as confusion, dysarthria, rigidity, gait disturbances, and hypokinesia, and is generally confirmed by marked hyperintensity of the globus pallidus by

magnetic resonance imaging (MRI) (Ono et al., 1995; Nagatomo et al., 1999). Symptomatic improvement and reversion to more normal T1-weighted MRI images following discontinuation of manganese supplementation support the diagnosis of manganism. While the parenteral route is thus involved in the increased risk of hypermanganesaemia during TPN, it is also involved in the recent rise in cases of manganism in adults associated with long-term intravenous use of illicit designer drugs. Sikk et al. (2007) described four cases of young adults presenting with symptoms of manganism (including impaired postural control, unsteady gait, manual dysfunction) in conjunction with long-term (7 months – 8 years) repeated use of the psychostimulant ephedrone. In two cases, drug use started as young as 17 and 19 years of age. The synthesis of ephedrone involved the oxidation of pseudoephedrine with potassium permanganate, which remained in the injected solution. Based on the drug injection history of two of the cases, and an analysis of the manganese content of a similarly synthesized ephedrone preparation, the authors estimate a total body burden of manganese corresponding to 900 and 500 mg/kg body weight compared to a normal body burden of 10-20 mg/kg (Schroeder et al., 1966). Cranial MRI of two individuals with exposures and symptoms similar to those described by Sikk et al. revealed hyperintense patterns in the globus pallidus indicating an abnormally high accumulation of manganese (Meral et al., 2007).

6.2 Chronic Toxicity to Infants and Children

Manganese is an essential nutrient, but it has toxic effects if exposure is excessive or prolonged, especially if exposure is by the inhalation route. A number of studies have reported correlations between early life exposure to excessive manganese and symptoms of impaired neurodevelopment as revealed on neurobehavioral tests and in poorer academic performance. In a prospective study of the neurobehavioral effects of *in utero* exposure to manganese, Takser et al. (2003) reported an inverse correlation between cord blood manganese at birth and three subscales of psychomotor development (McCarthy scales of children's abilities) measured at three years of age ($n = 126$): attention (partial $r = -0.33$, $p < 0.01$), nonverbal memory (partial $r = -0.28$, $p < 0.01$), and hand skills (partial $r = -0.22$, $p < 0.05$). The adverse effects of manganese on neurodevelopment in these children persisted after adjustment for gender and maternal education, although the effects of manganese on hand skills were only observed in boys. Similarly, Collipp et al. (1983) used a battery of tests, including cognitive and projective tests, psycho-educational evaluation, speech, language and hearing evaluations, and social services evaluations, to identify 16 children who were hyperkinetic and exhibited learning disabilities. In comparison with 44 normal children of the same age, significantly elevated levels of hair manganese (0.434 µg/g; measured at 8 years of age) were reported in children with learning disabilities and hyperactivity compared with normal children (0.268 µg/g) ($p < 0.05$). An association between poorer performance in school and elevated hair manganese (1.242 µg/g) has also been observed among children in China compared with children with more normal manganese levels (Zhang et al., 1995).

Wasserman et al. (2006) reported adverse effects of manganese in 10-year old children ($n = 142$) in Bangladesh who had been exposed to manganese in their drinking water (< 200, 200-499, 500-999, > 1,000 µg/l). Comparing the lowest and highest dose groups (< 200 vs. > 1,000 µg/l), significant decrements in intellectual function at 9.5-10.5 years of age were revealed in scores on the Wechsler Intelligence Scale for Children-III with increasing daily intake of manganese (full scale, $p < 0.0001$; performance, $p < 0.0001$; verbal, $p < 0.02$). The scores of children with

intermediate manganese exposures were also lower than those of the lowest dose group, but not significantly so. In this study, confounding by co-exposure to arsenic was limited by including only children whose drinking water contained < 10 µg As/l. Scores were adjusted for maternal education and intelligence, house type, television, child height and head circumference. Blood levels of manganese, arsenic and lead were also determined and added to the core model. In this case, only blood lead was correlated with decreased intellectual performance. However, in a simultaneous analysis of water manganese, water arsenic, and blood lead, the negative association between manganese water levels and intellectual function test scores remained (Full-Scale ß = -4.56, $p < 0.01$; Performance ß = -3.82, $p < 0.01$).

The uptake of metals into developing teeth provides a record of gestational exposure to manganese. In multiple regression analyses, after controlling for lead, high levels of manganese incorporated into teeth during the 20th week of gestation were positively correlated with behavioral disinhibition at 36 months of age ($R = 0.48$, $p < 0.01$) and, at 54 months, with impulsive errors on the Mirsky Continuous Performance Test ($R = 0.48$, $p < 0.01$) and the Children's Stroop Test ($R = 0.38$, $p < 0.01$). Positive correlations with manganese were also seen in ratings made by both parents and teachers of externalizing and attention problems on the Child Behavior Checklist in the 1st ($R = 0.40 – 0.47$, $p < 0.05$) and 3rd grades ($R = 0.38 – 0.48$, $p < 0.05$), and in the 3rd grade with the teachers' ratings on the Disruptive Behavior Disorders Scale ($R = 0.44$, $p < 0.05$), ADHD ($R = 0.48$, $p < 0.01$), and hyperactivity – impulsivity ($R = 0.55$, $p < 0.01$). In contrast, manganese levels in tooth enamel formed in the 62-64th week of gestation (i.e., postnatally) were correlated only with teachers' reports of externalizing behaviors in the 1st ($R= 0.40$, $p < 0.05$) and 3rd grades ($R = 0.57$, $p < 0.01$). It thus appears that high prenatal manganese exposure may adversely affect behaviors expressed postnatally. There was, however, no correlation between tooth manganese and cognitive ability as measured on the Woodcock-Johnson Psycho-Educational Battery (Ericson et al., 2007).

Subtle neurobehavioral effects were seen in a case report of a 10-year old boy exposed for five years to elevated manganese in the family's drinking water (Woolf et al., 2002). The boy's hair manganese was high (3,091 ppb vs normal reference < 260 ppb), as was that of his 16 year-old brother (1,988 ppb). Neuropsychological tests on the 10 year-old revealed intact global cognitive skills but striking deficits in visual and verbal memory (< 20th percentile in the Wide Range Assessment of Visual-motor Abilities). No obvious neurobehavioral problems were noted for either the parents or the older sibling.

As with adults, children receiving long-term parenteral nutrition are at greater risk of hypermanganesaemia. This is especially problematic since it is often the premature infants that require TPN. Among infants receiving parenteral nutrition containing supplemental manganese, MRI scans revealed bilaterally symmetrical hyperintense signals in the globus pallidus associated with movement disorders (dystonia and abnormal posturing) (Fell et al., 1996), and in basal ganglia, brainstem, and cerebellum associated with seizures (Komaki et al., 1999). In one infant these effects developed within eight months (Fell et al., 1996). While an abnormally high T1-weighted MRI signal suggests high brain manganese levels, especially when removal of manganese results in gradually diminishing signal intensity, it should be noted that not all patients with elevated brain manganese develop overt neurological symptoms. Kafritsi et al. (1998) reported on siblings receiving parenteral nutrition for 63 and 23 months that resulted in elevated blood manganese levels of 323 nmol/l and 516 nmol/l, respectively (normal = 73-210

nmol/l) and hyperintense signals in the globus pallidi. The signal intensity reverted to normal following cessation of manganese supplementation with no evidence of abnormal neurological development at three years of follow up. Whether either child had subclinical effects or effects that will manifest only later in life is unknown.

6.3 Animal Studies of Chronic Toxicity

Animal studies of the toxic effects of chronic manganese exposure have focused on altered neurobehavior and the effects of manganese on the associated brain structures. These studies indicate that differences in age at exposure, route, and chemical form of the metal are critical to the distribution of manganese, and the type and extent of the adverse effects.

Early life exposure to air borne manganese may occur by multiple routes: in utero via the mother, and perinatally via milk ingestion and inhalation. To examine the effects of these exposures on tissue concentrations of manganese in adult and young rats, adult male and female rats were exposed to $MnSO_4$ (0.05, 0.5, or 1 mg Mn/m^3; 1.05 µm GMD) or air 28 days prior to breeding, for up to 14 days during the mating period, during gestation days 0-19, and from one day post-partum through postnatal day (PND) 18 (Dorman et al., 2005a). Exposures were for 6 hr/day, 7 days/week. While these exposures did not affect maternal brain, lung, pancreas, or liver weights, the high dose (1 mg/m^3) was associated with decreased brain weights in pups on PND 14, female pups on PND 19, and male pups on PND 45. Measurements of manganese in the striatum, cerebellum, and olfactory bulb of neonatal rats on PND 19 showed statistically significant and dose-dependent increases relative to controls. On PND 18, maternal olfactory bulb, striatum, and cerebellum also had significantly ($p < 0.05$) elevated manganese from exposures to 0.5 mg/m^3 and above. Measurements made 27 days following exposure cessation on PND 45 showed that all tissue manganese levels had returned to control values. However, some pups on PND 45 still showed decreased brain weights suggesting that high early-life manganese exposure may result in prolonged alterations in brain size. By PND 63, all brain weights were at control values, but whether structural or functional deficits were present was not determined in this study.

Some consequences of early life exposure to manganese may not manifest until later in life. This appears to be the case for prenatal exposure to the manganese-containing fungicide Maneb (manganese ethylene-bis-dithiocarbamate), followed later in life by exposure to the pesticide paraquat and the development of symptoms of manganism. It has been reported previously (Thiruchelvam et al., 2000) that mice treated with Maneb twice a week for six weeks showed reduced motor activity immediately after treatment, with recovery of function in 24 hours. This effect was not seen with paraquat alone but was enhanced by co-exposure to paraquat. In these mice, co-exposure reduced tyrosine hydroxylase and dopamine transporter immunoreactivity in dorsal striatum. Similarly, only the combined Maneb/paraquat exposure decreased striatal tyrosine hydroxylase protein levels, caused reactive gliosis in dorsal-medial but not ventral striatum, and reduced tyrosine hydroxylase immunoreactivity and cell counts in the substantia nigra but not ventral tegmental area.

Barlow et al. (2004) extended these observations to early life susceptibility with the treatment of pregnant mice with Maneb, paraquat, or saline on gestation days 10-17. As adults, these mice received challenge exposures for eight days to either Maneb or paraquat on postnatal days 45-55.

Prior to the challenge exposures, locomotor activity was evaluated but no significant differences among groups were found. On the eighth day of challenge exposures, locomotor activity was depressed in all animals exposed to Maneb as adults, but recovered to control levels within one week of the last challenge exposure, except for males prenatally exposed to Maneb and subsequently exposed to paraquat as adults. These males showed a 95% reduction in locomotor activity, while similarly exposed females showed no effects. In the context of dopaminergic neurochemistry, neither prenatal exposure to Maneb alone nor adult exposure to paraquat alone caused significant change in either gender. However, compared to male controls and paralleling the locomotor effects, males receiving Maneb prenatally and paraquat as adults had 50% lower striatal dopamine levels, 35% lower 3,4-dihydroxyphenylacetic acid (DOPAC, a dopamine metabolite) levels, and 40% greater dopamine turnover. In the substantia nigra pars compacta, these males showed a loss of tyrosine hydroxylase-positive neurons of 30% compared with saline-treated males ($p < 0.001$), 30% compared with males receiving Maneb prenatally and saline as adults ($p < 0.001$), and 21% compared with males receiving paraquat as adults only ($p < 0.05$). The reduction in tyrosine hydroxylase positive neurons only occurred with Maneb followed by paraquat, not following Maneb alone. These results suggest that prenatal exposure to Maneb causes damage to the nigrostriatal region of the male brain that is only revealed in adulthood following another neurotoxic insult in the form of paraquat. While these experiments do not demonstrate that it is the manganese in Maneb that is responsible for the observed neurotoxicity, the types of toxicity described are similar to those observed for other manganese compounds. These experiments also do not address the potentially enhanced neurotoxicity associated with more continuous exposure to manganese as Maneb during prenatal to adult development. However, long-term exposure to Maneb among adult farm workers has been associated with the development of symptoms of Parkinson's Disease, characteristic of manganism (Ferraz et al., 1988; Meco et al., 1994). It should also be noted that while this experimental design emphasized the neurotoxicity of the sequential exposure to Maneb, then paraquat, it is possible that the deleterious effects of exposure to other neurotoxic substances during development or adulthood would also be enhanced by early life exposure to manganese-containing pesticides.

The relative sensitivity of neonatal and adult CD rats to manganese-induced neurotoxicity was studied by administering manganese dichloride orally to rats at doses of 0, 25, and 50 mg/kg per day (Dorman et al., 2000). Adults and pups were dosed for 21 consecutive days, and then were evaluated with behavioral tests such as pulse elicited startle response amplitude, and in terms of manganese levels in striatum, hippocampus, hindbrain, and cortex. Neonatal rats exposed at the highest level of manganese showed a statistically significant increase in amplitude of acoustic startle response. They also showed increases in brain levels of manganese. The results suggest that neonates may be at greater risk for manganese-induced neurotoxicity when compared to adults receiving high oral levels of manganese. The authors state that there are known pharmacokinetic processes that may relate to the increase in brain manganese concentration in neonatal rats including increased manganese absorption from the juvenile gastrointestinal tract, an incompletely formed blood-brain barrier, and a virtual absence of excretory mechanisms until weaning.

The effects of manganese inhalation on levels of the metal in various tissues has been explored in Rhesus monkeys. In a study by Dorman et al. (2006b), Rhesus monkeys, 20-24 months of age, inhaled manganese sulfate (60, 300 or 1,500 $\mu g/m^3$; 1.04, 1.07, 1.12 µm; GMD, respectively) 6

hours per day, 5 days per week for 13 weeks. At termination, tissue manganese levels were significantly ($p < 0.05$) elevated in all tissues examined, except testes, in animals exposed to the highest dose (1,500 µg/m^3). Even at the lowest dose (60 µg/m^3), manganese levels were significantly elevated in four of the eight brain regions examined, globus pallidus, putamen, white matter and cerebellum (Table 6.3.1). For comparison, the table includes manganese levels in these same brain regions reported below by Schneider et al. (2006) and Guilarte et al. (2006) for monkeys displaying neurobehavioral toxicity. To facilitate comparison with human occupational studies, an annual dose is also presented showing the calculated air concentration had the dose levels been spread over a period of one year. In the study by Roels et al (1992), used for the development of the REL (Section 8), a similar measure of the annualized exposure was calculated as the lifetime integrated respirable dose (LIRD). In the Roels study, neurotoxicity was observed in individuals with LIRDs in the range of 60 to 3,715 µg*yr/m^3, a range that overlaps the concentrations used by Dorman that resulted in brain levels associated with neurotoxicity by Schneider and Guilarte. Although the brain levels of manganese were not measured in workers showing neurotoxicity in the Roels study, these studies in primates provide support that the air concentrations to which the workers were exposed was sufficient to result in brain manganese levels with which neurotoxicity is associated in primates.

TABLE 6.3.1 MANGANESE LEVELS IN PRIMATE BRAIN AFTER INHALATION OR IV EXPOSURE

	Dorman et al., 2006			Schneider et al., 2006/
	Inhalation level			Guilarte et al., 2006
	60 µg/m^3	**300 µg/m^3**	**1,500 µg /m^3**	**3.26-4.89 mg Mn/kg***
Caudate	0.47 µg/g	0.69 µg/g	1.72 µg/g	1.18 µg/g
Putamen	0.58	0.75	1.81	1.50
Globus pallidus	0.80	1.28	2.94	3.30
White matter	0.25	0.39	0.87	0.57
Annual dose**	**15 µg*yr/m^3**	**75 µg*yr/m^3**	**375 µg*yr/m^3**	

* Neurotoxicity reported in monkeys with the indicated brain Mn levels.
* Roels reported neurotoxicity at an overlapping range: LIRD = 60 - 3,715 µg*yr/m^3

Neurobehavioral effects may be preceded by changes in brain chemistry. Such changes were studied in four female rhesus monkeys exposed in an inhalation chamber to 30 mg/m^3 respirable manganese dust for five hours/day, five days/week (Bird et al., 1984). After two years the animals were sacrificed and compared to unexposed controls. The exposed monkeys showed decreased dopamine in the caudate and globus pallidus, as well as a 60 to 80 percent increase in manganese levels in the basal ganglia of the brain. However, the exposed monkeys did not exhibit any of the movement disorders that are characteristic of Parkinson's disease.

In another study of the effects of manganese inhalation on neurotransmitters in rhesus monkeys (20-24 months old), Struve et al. (2007) found that subchronic (13 wk) exposure to manganese sulfate resulted in statistically significant increases in mean manganese concentrations in the pallidus and putamen at 0.06, 0.3, and 1.5 mg/m^3 (MMAD 1.73, 1.89, 2.12 µm) , and in the caudate at $\geq$ 0.3 mg/m^3. Marginally statistically significant ($p < 0.1$) changes in neurotransmitter levels were seen only at the highest manganese concentration (1.5 mg/m^3) in the globus pallidus

for GABA and 5-HIAA, and in the caudate for norepinephrine. This is consistent with the suggestion that manganese neurotoxicity derives in part from dis-regulation of GABA-ergic neurons (Fitsanakis et al., 2006), possibly related to the observed decreases in tyrosine hydroxylase and glutamine synthetase by manganese (Erikson et al., 2007a).

The distribution of manganese in primate brain, and its neurobehavioral and cognitive effects in 5-6 year old Cynomolgus macaques following weekly intravenous injection of $MgSO_4$ (10-15 $MgSO_4$ or 3.26-4.89 mg Mn/kg) for 39 weeks was investigated by Guilarte and associates. Neurobehavior, as rated on a modified Parkinsonian symptoms scale, activity levels measured with an activity monitor, and fine motor skills, assessed as the number of errors while trying to retrieve objects from wells of different sizes, all showed significant decrements ($p < 0.05$) at the end of the experiment compared with baseline (Guilarte et al., 2006a). Over this same period, stereotypical or compulsive-like behaviors, such as licking/biting fingers and grooming, significantly increased in frequency with manganese exposure ($p < 0.01$) (Schneider et al., 2006). The levels of manganese were significantly ($p < 0.05$) elevated in exposed monkeys compared to controls in the globus pallidus (3.3 µg/g tissue), caudate (1.18 µg/g), putamen (1.50 µg/g), and frontal white matter (0.57 µg/g) (Table 6.3.1). Imaging studies were performed at 128 days and 157 days after the start of manganese exposure, and included T-1 weighted magnetic resonance imaging (MRI), magnetic resonance spectroscopy (H-MRS), and positron emission tomography (PET). As assessed by PET, manganese decreased the ability of amphetamine to stimulate dopamine release in the striatum, apparently without the loss of dopaminergic terminals. The authors speculate that the inhibition of dopamine release may alter the excitability of nigrostriatal dopaminergic neurons and/or may alter dopamine compartmentalization. The former case may contribute to the behavioral symptoms while, in the latter case, the probability of dopamine oxidation and consequent neuronal damage may be increased (Guilarte et al., 2006a). Neuronal loss or dysfunction in these monkeys was suggested by a change in brain metabolites with chronic manganese exposure. Specifically, significant decreases in the N-acetylaspartate: creatinine ratio in parietal cortex ($p = 0.028$), and a near significant ($p = 0.055$) decrease in the white matter were observed.

Concern for the consequences of exposure to the combustion products of methylcyclopentadienyl manganese tricarbonyl (MMT) has fueled investigation of the bioaccumulation and neurobehavioral effects following subchronic exposure to manganese as the free metal, conjugates of sulfate and phosphate, and a mixture of the two conjugates. In a collection of related studies (Normandin et al., 2002; Salehi et al., 2003; Normandin et al., 2004; Tapin et al., 2005), young adult rats were exposed to aerosolized manganese or its conjugates for 6 hours/day, 5 days/week for 13 weeks at target levels of 30, 300, and 3,000 µg/m^3 (<1.55 – 6 µm MMAD). Following exposure, locomotor activity over a 36 hr period was recorded as resting time, distance traveled, and total ambulatory count. The animals were then sacrificed and the manganese levels in various tissues and brain regions measured. These studies consistently showed significant ($p < 0.05$) dose-dependent increases in manganese in the lungs for all forms of the metal. The highest dose of the manganese conjugates, separately or mixed, resulted in significantly ($p < 0.05$) elevated levels in all tissues except the liver, reflecting the liver's role in manganese homeostasis. The sulfate and phosphate conjugates were better assimilated into all extra-pulmonary tissues than was the un-conjugated metal (Normandin et al., 2004). Uptake of manganese into brain tissue was more efficient from the combined sulfate and phosphate exposure than from exposure to metallic manganese or the phosphate conjugate alone,

presumably due to the higher solubility of the sulfate conjugate. This difference was reflected in the significantly ($p < 0.05$) lower ambulatory count for the animals exposed to the conjugate mixture compared to controls (Normandin et al., 2004). For rats exposed to $MnSO_4$ alone, the total distance traveled in the locomotor studies increased at all manganese concentrations concomitant with an increase in total resting time, suggesting shorter bursts of activity. These rats also showed a dose-dependent decrease in total ambulatory counts over 36 hours, as well as a dose-dependent loss of cells in the globus pallidus and caudate putamen (Tapin et al., 2005). Similarly, the highest exposure to the Mn sulfate/phosphate mixture produced a significant increase in motor activity and a significant decrease in total ambulatory counts (Saleh et al., 2003). In contrast, no behavioral changes were noted with exposure to the phosphate conjugate (Normandin et al., 2002). Collectively these studies suggest that behavioral neurotoxic effects are associated with inhalation of manganese conjugates and that the sulfate conjugate is more toxic than the phosphate or metallic forms, consistent with its greater solubility.

In addition to neurotoxicity, pulmonary dysfunction may be associated with inhalation of manganese. In a subchronic chamber study, young, male rhesus monkeys were exposed to manganese sulfate at 0.06, 0.3, or 1.5 mg Mn/m^3 for 6 hours/day, 5 days/week, for 65 exposure days (Dorman et al., 2005b). The MMADs of the particles in these aerosols were 1.73, 1.89, and 2.12, respectively (1.04, 1.07, 1.12 µm GMD, respectively). Another set of monkeys, exposed to 1.5 mg Mn/m^3 by this regimen, was held for 45 or 90 days prior to evaluation. A third set was exposed to 1.5 mg Mn/m^3 and evaluated after 15 or 30 days of exposure. The evaluations included histopathological assessments of the lungs, and measurements of the manganese content of lungs and olfactory epithelium. Manganese levels were significantly elevated ($p < 0.05$) in olfactory epithelium with all exposures, and in lungs at exposures of 0.3 mg/m^3 and above. In animals exposed to 1.5 mg/m^3 and evaluated after 15 or 33 days, significantly ($p < 0.05$) elevated manganese levels were found in both olfactory epithelium and lungs, however, these levels returned to control levels 45 and 90 days after exposure was discontinued. Significant bronchiolitis and alveolar duct inflammation was seen only in the animals exposed to 1.5 mg Mn/m^3, but these effects were apparently reversible as they were no longer present at 45 and 90 days post exposure. Increased bronchus-associated-lymphoid tissue was also observed only with the highest exposure. Thus the inflammatory changes in small airways and increased manganese lung concentrations were only associated with the highest exposure levels used (1.5 mg Mn/m^3), and were apparently reversible following cessation of exposure, suggesting that the lungs are a less sensitive target for manganese toxicity than is the central nervous system.

The pulmonary toxicity of manganese, as measured by induction of an inflammatory response, is relatively high compared with a number of the transition metals found in particulate pollution. Intratracheal instillation of the sulfates of copper, vanadium, nickel, iron, zinc, and manganese (0.1, 1.0 µmol/kg) was used to assess the relative inflammatory potential of these metals in rats. Bronchoalveolar lavage (BAL) at 4, 16 and 48 hours following exposure provided the medium in which markers of inflammation were measured (Rice et al., 2001). Lactate dehydrogenase activity and total protein levels in the lavage fluid, used as general indicators of toxicity, showed copper at 1 µmol/kg to be the most toxic at all time points, followed by nickel and manganese. Whereas with copper LDH activity peaked at 16 hours post-exposure, then declined by 48 hours, manganese-induced LDH activity was significantly ($p < 0.05$) elevated at all time points and continued to increase with time. Similarly, the numbers of leukocytes recovered from BAL fluid were highest for manganese compared with the other metals at 16 and 48 hours. At these same

time points, neutrophilia was seen at the low dose only with copper, while at the higher dose, manganese was the most potent. Significant eosinophilia was observed for manganese, copper, iron, and nickel at 16 and 18 hours, but eosinophil numbers were the highest with manganese by two to three-fold. Lymphocyte levels were not elevated by metal treatment except with low-dose copper at 16 hours, and high dose manganese at 48 hours. Thus manganese and copper were found to be the most proinflammatory of the metals tested but presumably by different signaling pathways. The effects with copper tended to appear earlier and at lower exposure levels, while manganese was more effective at stimulating the appearance of immune cells at a higher dose and later time points.

6.4 Dietary Exposure to Manganese

Newborns and infants may be exposed to more manganese in their diets than are adults. Infant formulas based on cow's milk have about 16 times more manganese than human milk (Dorner et al., 1989). Soy based formulas have even higher levels of manganese – about 40 times the manganese of human milk (Tran et al., 2002a; Tran et al., 2002b). Formula usage can lead to significantly elevated body burdens of manganese. For example, the hair manganese in normal infants at birth was reported to be 0.19 µg/g hair and, in breast-fed infants, increased to 0.330 µg/g at four months of age. By comparison, hair manganese levels in infants on a formula diet reached 0.965 µg/g at six weeks of age, and 0.685 µg/g at four months (Collipp et al., 1983). In addition, infants can have a less varied diet than adults and may consume more of certain foods that are high in manganese (e.g., sweet potatoes, 2.6 mg/cup; spinach, 1.8 mg/cup; oatmeal, 1.4 mg/cup; (NWU, 2006)).

6.5 Nutritional Requirement

Manganese is an essential nutrient involved in the formation of bone, and in amino acid, cholesterol, and carbohydrate metabolism (FNB, 2004). It is required in a number of metalloenzymes, including arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and superoxide dismutase (FNB, 2004). Levels of manganese in adult tissues are maintained at stable levels by homeostatic mechanisms that involve regulation of both uptake and excretion (Aschner and Aschner, 2005). Manganese homeostasis is not maintained in newborn infants, and it is not clear how long it takes for it to develop (FNB, 2004); homeostasis in mice takes 17 to 18 days to become effective (Fechter, 1999). Rat pups born to manganese-exposed mothers (dosed with 2000 ppm Mn in drinking water throughout pregnancy and for 11 days of lactation) have seven times the manganese (whole body) as controls (Kostial et al., 2005). By weaning (11 days after birth) the manganese concentration in both groups is virtually the same, indicating that in rat pups manganese homeostasis may begin shortly after birth and become effective by weaning (Kostial et al., 2005).

Adequate intakes (AI) of manganese have been established by the Food and Nutrition Board of the Institute of Medicine (FNB, 2004). They are given in Table 6.5.1 below. This table also contains tolerable upper intake levels (UL) for manganese consumption. It is of note that in many cases the UL is not very far above the AI level. For children one to three years of age the UL is less than twice the AI.

The AI for infants 0 to 6 months was set based on the amount of manganese in human milk and the average amount of milk consumed. There are no reports of nursing infants showing any symptoms of manganese deficiency (FNB, 2004). The AI for infants 7 to 12 months of age is based on the manganese content of a typical diet including human milk and other foods. This AI is much higher than the one for infants 0 to 6 months because the manganese content of other foods is generally much higher than the manganese content of human milk (FNB, 2004).

TABLE 6.5.1 ADEQUATE INTAKES AND TOLERABLE UPPER INTAKE LEVELS FOR MANGANESE FOR DIFFERENT AGE GROUPS

Group	Adequate Intake (AI) (mg/day)	Tolerable Upper Intake Level (UL) (mg/day)
Infants, 0-6 months	0.003	"not possible to establish"
Infants, 7-12 months	0.6	"not possible to establish"
Children, 1-3 years	1.2	2
Children, 4-8 years	1.5	3
Boys, 9-13 years	1.9	6
Boys, 14-18 years	2.2	9
Girls, 9-13 years	1.6	6
Girls, 14-18 years	1.6	9
Men, 19 to >70 years	2.3	11
Women, 19 to >70 years	1.8	11
Pregnant women, 14-18 yrs	2	9
Pregnant women, 19-50 yrs	2	11
Lactating mothers, 14-18 years	2.6	9
Lactating mothers, 19-50 years	2.6	11

6.6 Potential for Differential Effects in Children

Infants and children may be more susceptible than adults to manganese toxicity for the following toxicodynamic and toxicokinetic reasons:

1. As noted in the previous section, manganese exposures in childhood are associated with impaired neurodevelopment including decrements in intellectual function. Thus a major toxicodynamic factor that differs between adults and children, namely development of the central nervous system, presents hypersensitive targets for toxicity in the developing infant and child.
2. Early life manganese exposures may predispose to manifestations of neurological damage in adulthood following subsequent exposure to environmental toxicants (Barlow et al., 2004).
3. Newborns absorb and retain more manganese from the gastrointestinal tract than do adults (Dorner et al., 1989; Davis et al., 1993).
4. The liver of newborns has not yet developed the ability to maintain safe levels of manganese in the bloodstream and brain tissues by excreting excess manganese in the bile, i.e., homeostasis of manganese has not yet developed (Miller et al., 1975).
5. Some infant formulas and foods are high in manganese. Soy formula may contain 200-300 µg Mn/l compared with 6 µg Mn/l for breast milk (Tran et al., 2002a,b). High dietary intake, combined with inhalation exposure, may put infants at greater risk of manganese toxicity.
6. The newborn's brain is still developing, myelination is incomplete, and the blood-brain barrier is not fully formed (Chan et al., 1992). These conditions facilitate manganese uptake into the central nervous system and increase the risk of attaining toxic levels.
7. Modeling of the inhalation dosimetry of particles (0.001-10 µm), comparing infants (3 mo) and adults, in four regions of the respiratory tract (extra-thoracic, tracheo-upper bronchi, bronchiolar, pulmonary), suggests that differences in the dose per unit surface area between neonates and adults are dependent on particle size and respiratory tract region (Ginsberg et al., 2005). These differences are most pronounced in the pulmonary and bronchiolar regions for ultrafine particles in the 0.1 to 0.001 µm range where neonates experience a 2-4 fold higher particle dose. In addition, infants and young children experience overall higher deposition of particles than adults.
8. Manganese absorption from the nose (Thompson et al., 2007), lungs (Brain et al., 2006), and intestinal tract (Erikson et al., 2002) is enhanced by low iron levels, a condition more prevalent among children than adults. Uptake directly from the nose or from the lungs bypasses first pass through the liver.

7. Developmental and Reproductive Toxicity

While data are scarce on the developmental effects of perinatal manganese exposure in humans, rats exposed to supplemental manganese (50, 250, 500 µg/day) beginning at birth show decreased dopamine in the striatum and poorer performance on behavioral tests (Tran et al., 2002b). This is consistent with studies examining manganese levels in various brain regions following developmental exposure. Female rats were exposed to $MnCl_2$ in drinking water (10 mg/ml) from the time of mating through weaning. The female offspring were similarly treated until sacrifice at 5, 10, 22, or 120 days postpartum (Chan et al., 1992). These time points represented the early postnatal period (day 5), the period of active myelination (days 10- 22), and sexual maturation (days 22-120). As shown in Table 7.1, manganese levels in all regions of the 5-day-old brain, except the cerebellum, are significantly elevated relative to unexposed controls. During the period of myelination (days 10-22), the manganese concentrations decreased. However, compared with controls, the concentrations in the treated rats were 2-13-fold higher, with the greatest difference in the striatum, followed by the midbrain. The differences in levels between groups decreased through sexual maturation. These data suggest that manganese distribution in the developing brain is heterogeneous and age-dependent, with the striatum and midbrain as potentially more susceptible regions for metal accumulation with high exogenous exposure.

TABLE 7.1 BRAIN REGIONAL MN CONCENTRATIONS (µG/G WET WT.) FR. CHAN ET AL. (1992)

Brain Region		Postnatal Age (days) 5	10	22	120
Hypothalamus	Control	2.50 ± 0.33[c]	0.52 ± 0.05[c]	0.42 ± 0.01[c]	0.30 ± 0.03
	Mn	4.52 ± 0.72[c,,e]	0.99 ± 0.11[c,e]	2.23 ± 0.20[c,e]	1.11 ± 0.22[e]
Cerebellum	Control	5.73 ± 0.28[c]	3.97 ± 0.19[c]	0.47 ± 0.16	0.38 ± 0.05
	Mn	4.95 ± 0.95[c]	6.16 ± 0.11[c,e]	1.32 ± 0.09[b,e]	0.94 ± 0.07[e]
Pons & medulla	Control	9.56 ± 1.16[c]	4.73 ± 0.50[c]	0.42 ± 0.01[b]	0.35 ± 0.04
	Mn	13.86 ± 0.53[c,e]	5.00 ± 0.28[c]	1.46 ± 0.05[b,e]	1.07 ± 0.23[e]
Striatum	Control	12.05 ± 0.10[c]	1.78 ± 0.10[c]	0.12 ± 0.02[c]	0.24 ± 0.03
	Mn	12.86 ± 0.54[c,d]	3.72 ± 0.13[c,e]	1.57 ± 0.24[b,e]	1.13 ± 0.24[e]
Midbrain	Control	1.96 ± 0.27[c]	1.51 ± 0.08[c]	0.19 ± 0.04[c]	0.38 ± 0.07
	Mn	6.43 ± 0.51[c,e]	2.49 ± 0.03[c,e]	2.15 ± 0.04[b,e]	1.35 ± 0.32[e]
Cerebral cortex	Control	0.85 ± 0.20[c]	1.15 ± 0.10[c]	0.19 ± 0.04[b]	0.34 ± 0.07
	Mn	4.42 ± 0.21[c,e]	2.56 ± 0.05[c,e]	1.39 ± 0.16[c,e]	0.62 ± 0.16[d]

[a] Values are the means ± SD of 6-10 female rats [b] $P < 0.05$ compared to day 120 by ANOVA
[c] $P < 0.01$ compared to day 120 by ANOVA [d] $P < 0.01$ compared to age-matched controls by t-test
[e] $P < 0.001$ compared to age-matched controls by t-test

In children on long-term parenteral nutrition resulting in blood manganese levels of 615-1840 nmol/l (vs reference range of 72-210 nmol/l), elevated manganese levels have been seen in globus pallidus and subthalamic nuclei (Fell et al., 1996), suggesting an enhanced potential for

neurological damage. This is consistent with the decrements in intellectual function in children exposed to manganese in drinking water reported by Wasserman et al. (2006).

The effects of manganese on reproduction in humans have been reported in epidemiological studies of workers with occupational exposure to manganese. The results have been mixed with Gennart et al. (1992) reporting no effect on fertility among workers exposed to a median manganese dust level of 0.71 mg/m^3, while those exposed to 0.07-8.61 mg/m^3 (geometric mean 0.94 mg/m^3) in a study by Lauwerys et al. (1985) sired a statistically significant lower number of children during the period of paternal exposure. However, workers in the Gennart et al. study were exposed to the relatively insoluble manganese oxide and had mean urine manganese levels of 0.82 µg/g creatinine. By comparison, the workers in the study by Lauwerys et al. were exposed to the more soluble manganese salts in addition to the oxide, and had mean urinary manganese levels of 4.37 µg/g creatinine. Thus the differences in the effects of manganese on reproduction reported in these two studies may be due to the significant differences in manganese exposures.

Adverse changes in reproductive parameters and behaviors have been seen in studies of rodents exposed to high levels of manganese. In immature female rats (23 days old), manganese (1-25 µg $MnCl_2$) introduced into the third ventricle of the brain significantly and dose-dependently stimulated the release of luteinizing hormone (LH). This effect was apparently at the level of the hypothalamus as pretreatment with the LH releasing hormone (LHRH) receptor antagonist, acyline, prior to manganese exposure blocked the release of LH (Pine et al., 2005). These authors further reported that serum LH, follicle stimulating hormone, and estradiol were all elevated by 29 days of age in rats that had received $MnCl_2$ by gavage starting on postnatal day 12. In these animals manganese altered the timing of reproductive events resulting in a significantly ($p < 0.001$) earlier onset of puberty as measured by vaginal opening.

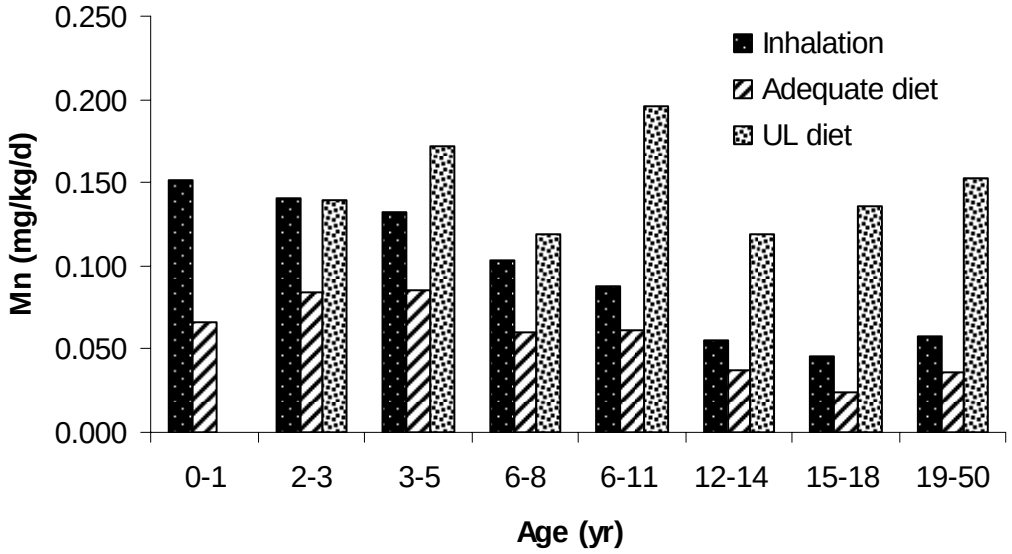
In adult male rats, exposure to 1,000 ppm manganese sulfate in drinking water for 12 weeks significantly suppressed sexual performance compared with controls as measured by prolonged ejaculatory latencies ($p < 0.001$), and increased post-ejaculatory intervals ($p < 0.05$). Displays of aggressive behaviors (lateralizations, boxing bouts, and fights with stud males) were also reduced ($p < 0.001$). The extent to which the altered behaviors represent neurological effects versus effects on testes and androgen production is not clear. However, among females mated to the manganese-treated males, the total number of resorptions was significantly increased ($p < 0.025$), suggesting a testicular effect. This is supported by a significant ($p < 0.001$) reduction in absolute and relative testes weights, and absolute seminal vesicle weights among manganese-exposed males (Bataineh et al., 1998). An effect of manganese on male reproductive organs was also investigated in mice following 43 days of oral manganese acetate (7.5 – 30 mg/kg/d) (Ponnapakkam et al., 2003). Unlike the study with rats above, there was no significant change in testicular weight or pathology with manganese exposure in the mice. Nor was there evidence of abnormal mating behavior. However, epididymal weights were significantly lower ($p < 0.05$) and there was a significant ($p < 0.001$) dose-dependent decrease in sperm number and motility.

The available data suggest that manganese is a reproductive toxicant in animals (both males and females) albeit at relatively high doses. Neurobehavioral toxicity manifests at levels encountered in the environment (Wasserman et al., 2006). Whether this decrement in intellectual function represents a true developmental effect with permanent consequences is not clear.

8. Derivation of Reference Exposure Levels

The determination of safe exposure levels to manganese is complicated by its status as an essential nutrient. However, as described above, inhalation of manganese results in a qualitatively and quantitatively different exposure compared to oral intake, with inhalation resulting in more rapid uptake and higher blood and brain levels. While dietary manganese levels moderate intestinal absorption of manganese, there appears to be no effect of dietary manganese on the pharmacokinetics of inhaled manganese (Dorman et al., 2002b). To provide perspective on potential manganese exposure from inhalation relative to the suggested upper limits for age-dependent dietary intake, we compared the potential manganese internal dose from inhalation with that from the recommended dietary levels for the various age groups indicated (Fig. 8.1). For this comparison, we used an air level of manganese of 0.215 mg Mn/m^3, the average level of respirable manganese dust to which workers were exposed in an occupational study of the effects of manganese exposure (Roels et al., 1992). It thus represents a high but real world exposure level associated with neurotoxicity. Adequate dietary intake and the recommended upper limit levels, above which toxic effects may be observed, are those set by the Food and Nutrition Board (FNB, 2004). The data for inhalation represent the amount of manganese taken up by the different groups, age-adjusted for average breathing rates per kilogram body mass per day (OEHHA, 2000; Arcus-Arth and Blaisdell, 2007). For this example, we assumed that uptake from the lungs is 100% of the inhaled manganese, and that absorption from dietary intake is 41% for 0-1 yrs, 10% for 2-3 yrs, and 5% for all other ages. The inhaled manganese becomes the age-specific breathing rate multiplied by the air concentration. We compared the inhaled dose to the internal dose of manganese expected from an adequate diet, and from intake at the upper limit. This analysis suggests that among neonates and children through age 8, the manganese uptake from inhalation of this high level of manganese would substantially exceed the adequate dietary amount and may approach or exceed the levels beyond which toxicity may be expected. Therefore, compared with adults at the same exposure level, a child's manganese inhalation is a larger proportion of the maximum recommended levels. This is due to the higher breathing rates, lower body mass, and greater absorption of manganese by children. Thus, for comparable air exposures, children are more at risk for exceeding safe levels than are adults.

FIGURE 8.4 INTERNAL MANGANESE DOSE FROM INHALATION AND DIET BY AGE



Age- and breathing rate-adjusted internal manganese dose following chronic inhalation of 0.215 mg/m^3 manganese (Inhalation), consumption of recommended minimum daily intake (Adequate diet), or upper limit level beyond which toxic effects may be observed (UL diet). Data expressed as mg Mn/kg body weight/day.

8.1 Manganese Acute Reference Exposure Level

Acute Reference Exposure Levels (RELs) are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document (TSD)). Pulmonary damage and inflammation are the principal endpoints associated with acute inhalation exposure to manganese. However, at present the database is insufficient to allow the development of an acute REL for manganese based on inhalation studies. No studies were located that reported dose-response data for acute inhalation exposures, nor was it possible to determine both LOAELs and NOAELs from the available data.

8.2 Manganese 8-Hour Reference Exposure Level

Study	Roels et al., 1992
Study population	92 workers in a battery plant
Exposure method	Inhalation of workplace air
Exposure continuity	
Exposure duration	8 hr/day, 0.2-17.7 yr (mean 5.3 yr)
Critical effects	Impaired neurobehavior: visual reaction time, eye-hand coordination, hand steadiness
NOAEL	not observed
Benchmark concentration	72 μg/m^3
Time-adjusted exposure	51 μg/m^3 (72*5/7)
LOAEL uncertainty factor (UF_L)	Not applicable
Subchronic uncertainty factor (UFs)	√10 (default 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10 (greater absorption and lung deposition in children)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children to neurotoxicity)
Cumulative uncertainty factor	300
Reference Exposure Level	**0.17 μg/m^3**

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the TSD).

The proposed 8-hr REL for manganese is 0.17 μg/m^3 based on impairment of neurobehavioral function in humans in the occupational study of Roels et al. (1992) described in Section 6.1. Data on the lifetime integrated exposure to respirable dust (LIRD) for each of the 92 workers and whether or not their response scores were abnormal on each of the three tests (visual reaction time, eye-hand coordination, and hand steadiness), were originally compiled by Dr. Roels, and were provided to OEHHA by Dr. J. Michael Davis of the US EPA. Abnormal scores were defined as those values that exceeded the 95th percentile value of the parameter as estimated from the cumulative frequency curves of the control group as in Roels et al. (1982). The LIRD was estimated for each worker based on the current airborne manganese concentration characteristic of each job multiplied by the number of years the worker had spent in that job. The result for each job held by an individual worker was then summed to obtain the LIRD for the individual. In our analysis, using US EPA's BMDS version 1.4.1b software, the LIRD (in μg/m^3 x yr), divided by the total years of manganese exposure for each individual, was compared with his performance on each of the three tests (scored as 0 if the test was normal and 1 if abnormal). In this treatment, the group size is 1. Eye-hand coordination (EHC) and hand steadiness (HST) are the two most critical endpoints; the incidence of changes in the visual reaction time in the study population was consistently lower. For both these endpoints, the best fitting models were the

Probit and Logistic models; the quality of fit was not distinguishable on statistical grounds. The lower 95% confidence bound benchmark confidence level ($BMCL_{05}$) for the Probit fit to the EHC data was selected as the most health protective value among these relatively closely spaced results.

Eye Hand Coordination (EHC)					
Model	BMC_{05}	$BMCL_{05}$	BMC/BMCL	P for fit	AIC
Probit	97	**72**	1.4	0.3797	93.9124
Logistic	105	78	1.4	0.3874	93.8307
Hand Steadiness (HST)					
Model	BMC_{05}	$BMCL_{05}$	BMC/BMCL	P for fit	AIC
Probit	162	115	1.4	0.2201	68.2214
Logistic	175	125	1.4	0.2389	67.9531

This analysis yielded a $BMCL_{05}$ of 72 µg Mn/m^3. A time correction was applied since the workers in the study were only exposed 5 days per week, whereas the 8-hour REL is designed to protect against daily exposures, as noted in the TSD. A cumulative UF of 300 was applied, comprising a √10 for subchronic to chronic conversion (average exposure duration = 5.3 yr; Section 4.4.6 of the TSD), and 10 each for intraspecies toxicokinetic (UF_{H-k}) and toxicodynamic (UF_{H-d}) uncertainty, resulting in an 8-hour REL of 0.17 µg Mn/m^3. This REL is based on healthy adult male workers with adjustments for the potentially greater susceptibility of children. The intraspecies UF_{H-k} of 10 was chosen in part to reflect the 3-4-fold greater deposition of inhaled particulates in the 1-10 µm size range in the lungs of neonates relative to adults exposed to similar particulate levels in ambient air (Ginsberg et al., 2005). In addition, neonates and infants more efficiently absorb and retain manganese than do adults (Dorner et al., 1989).

A UF_{H-d} of 10 is used to address the expectation that the still-developing brain of newborn and infant children is more sensitive to the effects of manganese and that injuries to the nervous system during development are anticipated to have lasting effects.

The development of these RELs is based on a benchmark concentration analysis of data from an occupational study as described above. An alternative approach involves the use of physiologically-based pharmacokinetic (PBPK) models, the development of which in rats has been described in a recent series of papers by workers at the Chemical Industry Institute of Toxicology , now the Hamner Institutes for Health Sciences (Teeguarden et al., 2007a; Teeguarden et al., 2007b; Teeguarden et al., 2007c); (Nong et al., 2008). While these papers represent significant progress in the modeling of manganese pharmacokinetics, they have yet to be extended to and validated in humans or non-human primates. For this reason, they were not used for the estimation of these REL values.

8.3 Manganese Chronic Reference Exposure Level

Study	Roels et al., 1992
Study population	92 workers in a battery plant
Exposure method	Inhalation of workplace air
Exposure continuity	
Exposure duration	8 hr/day, 0.2-17.7 yr (mean 5.3 yr)
Critical effects	Impaired neurobehavior: visual reaction time, eye-hand coordination, hand steadiness
NOAEL	not observed
Benchmark concentration	72 µg/m^3
Time-adjusted exposure	26 µg/m^3 (72 µg/m^3 x 10/20 x 5/7)
LOAEL uncertainty factor (UF_L)	Not applicable
Subchronic uncertainty factor (UFs)	√10 (default 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10 (greater absorption and lung deposition in children)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children to neurotoxicity)
Cumulative uncertainty factor	300
Reference Exposure Level	**0.09 µg/m^3**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from continuous lifetime exposures (see Section 7 in the Technical Support Document).

The proposed chronic REL for manganese of 0.09 µg/m^3 is based on impairment of neurobehavioral function in humans in the occupational study of Roels et al. (1992). The benchmark dose approach was used, as described above for the 8-hour REL, to derive a $BMCL_{05}$ of 72 µg/m^3. This corresponded to a time-adjusted concentration of 26 µg/m^3 (based on an 8 hour TWA occupational exposure to 10 m^3 manganese-contaminated air per day out of 20 m^3 total air inhaled per day over 5 days/week).

A cumulative UF of 300 was used, comprising a √10 for subchronic to chronic conversion (average exposure duration = 5.3 yr; Section 4.4.6 of the TSD), and 10 each for intraspecies toxicokinetic (UF_{H-k}) and toxicodynamic (UF_{H-d}) uncertainty. This REL is based on healthy adult male workers with adjustments for the potentially greater susceptibility of children. The intraspecies UF_{H-k} of 10 was chosen in part to reflect the 3-4-fold greater deposition of inhaled particulates in the 1-10 µm range in the lungs of neonates relative to adults exposed to similar particulate levels in ambient air (Ginsberg et al., 2005). In addition, neonates and infants more efficiently absorb and retain manganese than do adults (Dorner et al., 1989). It should also be noted that the effects reported in the Roels study were to a relatively insoluble form of

manganese, MnO_2. As shown in Table 4.1 above, exposures to similar levels of the more soluble $MnCl_2$ by the same route result in higher manganese brain levels.

A UF_{H-d} of 10 is used to address the expectation that the still-developing brain of newborn and infant children is more sensitive to the effects of manganese and that injuries to the nervous system during development are anticipated to have lasting effects. This REL was developed with specific consideration of the potentially greater susceptibility of children to manganese neurotoxicity. For comparison, the RfC for chronic manganese inhalation developed by the US EPA is 0.05 $\mu g/m^3$ (U.S.EPA, 1993) and is also based on Roels et al. (1992).

As described in Section 6.3 above, the studies by Dorman et al. (2006), Guilarte et al. (2006) and Schneider et al. (2006) report comparable post-controlled exposure manganese levels in the same regions of the brains of Rhesus monkeys, albeit using different experimental protocols. Based on the inhalation data from Dorman et al., it is possible to extrapolate an air manganese concentration at which the neurotoxicity reported by Guilarte and Schneider would have been expected. After adjustment for the differences in exposure duration, the projected air manganese levels from Dorman corresponding to the toxicity-associated region-specific brain levels reported by Guilarte are 75 $\mu g/m^3$ inhaled concentration for the caudate, 98 $\mu g/m^3$ for putamen, 150 $\mu g/m^3$ for globus pallidus, and 73 $\mu g/m^3$ for white matter. While the differences in the exposure regimens among these studies precludes using them to derive a REL, it is relevant and supportive that this range of exposure subsumes the LOAEL for neurotoxic effects in humans reported by Lucchini et al. (1999) of 97 $\mu g\ Mn/m^3$, and the point of departure for the REL based on the Roels et al. study of 72 $\mu g\ Mn/m^3$. Thus, these studies of non-human primates support the effect level upon which the REL is based.

8.4 Manganese as a Toxic Air Contaminant

In view of the potential for higher exposure in children than adults coupled with a lower ability to regulate manganese, and enhanced neurodevelopmental susceptibility leading to differential impacts in infants and children identified in Section 6.2.1, OEHHA recommends that manganese be identified as a toxic air contaminant which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

9. References

Adkins B, Jr., Luginbuhl GH and Gardner DE (1980). Acute exposure of laboratory mice to manganese oxide. Am Ind Hyg Assoc J 41(7): 494-500.

Andersen ME, Gearhart JM and Clewell HJ, 3rd (1999). Pharmacokinetic data needs to support risk assessments for inhaled and ingested manganese. Neurotoxicology 20(2-3): 161-71.

Archibald FS and Tyree C (1987). Manganese poisoning and the attack of trivalent manganese upon catecholamines. Arch Biochem Biophys 256(2): 638-50.

Arcus-Arth A and Blaisdell RJ (2007). Statistical distributions of daily breathing rates for narrow age groups of infants and children. Risk Anal 27(1): 97-110.

Aschner JL and Aschner M (2005). Nutritional aspects of manganese homeostasis. Mol Aspects Med 26(4-5): 353-62.

Aschner M (2000). Manganese: brain transport and emerging research needs. Environ Health Perspect 108 Suppl 3: 429-32.

Aschner M, Erikson KM and Dorman DC (2005). Manganese dosimetry: species differences and implications for neurotoxicity. Crit Rev Toxicol 35(1): 1-32.

Barbeau A (1984). Manganese and extrapyramidal disorders (a critical review and tribute to Dr. George C. Cotzias). Neurotoxicology 5(1): 13-35.

Barlow BK, Richfield EK, Cory-Slechta DA and Thiruchelvam M (2004). A fetal risk factor for Parkinson's disease. Dev Neurosci 26(1): 11-23.

Bataineh H, Al-Hamood MH and Elbetieha AM (1998). Assessment of aggression, sexual behavior and fertility in adult male rat following long-term ingestion of four industrial metals salts. Hum Exp Toxicol 17(10): 570-6.

Bergstrom R (1977). Acute pulmonary toxicity of manganese dioxide. Scand J Work Environ Health 3 Suppl 1: 1-41.

Bird ED, Anton AH and Bullock B (1984). The effect of manganese inhalation on basal ganglia dopamine concentrations in rhesus monkey. Neurotoxicology 5(1): 59-65.

Boojar MM and Goodarzi F (2002). A longitudinal follow-up of pulmonary function and respiratory symptoms in workers exposed to manganese. J Occup Environ Med 44(3): 282-90.

Bouchard M, Mergler D, Baldwin M, Panisset M, Bowler R and Roels HA (2007a). Neurobehavioral functioning after cessation of manganese exposure: a follow-up after 14 years. Am J Ind Med 50(11): 831-40.

Bouchard M, Mergler D, Baldwin M, Panisset M and Roels HA (2007b). Neuropsychiatric symptoms and past manganese exposure in a ferro-alloy plant. Neurotoxicology 28(2): 290-7.

Bowler RM, Roels HA, Nakagawa S, Drezgic M, Diamond E, Park R, Koller W, Bowler RP, Mergler D, Bouchard M, Smith D, Gwiazda R and Doty RL (2007). Dose-effect relationships between manganese exposure and neurological, neuropsychological and pulmonary function in confined space bridge welders. Occup Environ Med 64(3): 167-77.

Brain JD, Heilig E, Donaghey TC, Knutson MD, Wessling-Resnick M and Molina RM (2006). Effects of iron status on transpulmonary transport and tissue distribution of Mn and Fe. Am J Respir Cell Mol Biol 34(3): 330-7.

Brenneman KA, Wong BA, Buccellato MA, Costa ER, Gross EA and Dorman DC (2000). Direct olfactory transport of inhaled manganese ((54)MnCl(2)) to the rat brain: toxicokinetic investigations in a unilateral nasal occlusion model. Toxicol Appl Pharmacol 169(3): 238-48.

Calne DB, Chu NS, Huang CC, Lu CS and Olanow W (1994). Manganism and idiopathic parkinsonism: similarities and differences. Neurology 44(9): 1583-6.

CARB. (2005a). *California Toxics Inventory for 2004*. California Air Resources Board. http://www.arb.ca.gov/toxics/cti/cti.htm.

Cawte J (1985). Psychiatric sequelae of manganese exposure in the adult, foetal and neonatal nervous systems. Aust N Z J Psychiatry 19(3): 211-7.

Chan AW, Minski MJ, Lim L and Lai JC (1992). Changes in brain regional manganese and magnesium levels during postnatal development: modulations by chronic manganese administration. Metab Brain Dis 7(1): 21-33.

Collipp PJ, Chen SY and Maitinsky S (1983). Manganese in infant formulas and learning disability. Ann Nutr Metab 27(6): 488-94.

CRC (2005). CRC Handbook of Chemistry and Physics. Boca Raton (FL): CRC Press.

Crump KS (2000). Manganese exposures in Toronto during use of the gasoline additive, methylcyclopentadienyl manganese tricarbonyl. J Expo Anal Environ Epidemiol 10(3): 227-39.

Crump KS and Rousseau P (1999). Results from eleven years of neurological health surveillance at a manganese oxide and salt producing plant. Neurotoxicology 20: 273-86.

Davis CD, Malecki EA and Greger JL (1992). Interactions among dietary manganese, heme iron, and nonheme iron in women. Am J Clin Nutr 56(5): 926-32.

Davis CD, Zech L and Greger JL (1993). Manganese metabolism in rats: an improved methodology for assessing gut endogenous losses. Proc Soc Exp Biol Med 202(1): 103-8.

Dobson AW, Weber S, Dorman DC, Lash LK, Erikson KM and Aschner M (2003). Oxidative stress is induced in the rat brain following repeated inhalation exposure to manganese sulfate. Biol Trace Elem Res 93(1-3): 113-26.

Dodd CA, Ward DL and Klein BG (2005). Basal ganglia accumulation and motor assessment following manganese chloride exposure in the C57BL/6 mouse. Intl J Toxicol 24(6): 389-397.

Dorman DC, Brenneman KA, McElveen AM, Lynch SE, Roberts KC and Wong BA (2002a). Olfactory transport: a direct route of delivery of inhaled manganese phosphate to the rat brain. J Toxicol Environ Health A 65(20): 1493-511.

Dorman DC, McElveen AM, Marshall MW, Parkinson CU, James RA, Struve MF and Wong BA (2005a). Tissue manganese concentrations in lactating rats and their offspring following combined in utero and lactation exposure to inhaled manganese sulfate. Toxicol Sci 84(1): 12-21.

Dorman DC, McManus BE, Marshall MW, James RA and Struve MF (2004). Old age and gender influence the pharmacokinetics of inhaled manganese sulfate and manganese phosphate in rats. Toxicol Appl Pharmacol 197(2): 113-24.

Dorman DC, Struve MF, Gross EA, Wong BA and Howroyd PC (2005b). Sub-chronic inhalation of high concentrations of manganese sulfate induces lower airway pathology in rhesus monkeys. Respir Res 6: 121.

Dorman DC, Struve MF, James RA, Marshall MW, Parkinson CU and Wong BA (2001). Influence of particle solubility on the delivery of inhaled manganese to the rat brain: manganese sulfate and manganese tetroxide pharmacokinetics following repeated (14-day) exposure. Toxicol Appl Pharmacol 170(2): 79-87.

Dorman DC, Struve MF, Marshall MW, Parkinson CU, James RA and Wong BA (2006b). Tissue Manganese Concentrations in Young Male Rhesus Monkeys following Subchronic Manganese Sulfate Inhalation. Toxicol Sci 92(1): 201-10.

Dorman DC, Struve MF, Vitarella D, Byerly FL, Goetz J and Miller R (2000). Neurotoxicity of manganese chloride in neonatal and adult CD rats following subchronic (21-day) high-dose oral exposure. J Appl Toxicol 20(3): 179-87.

Dorman DC, Struve MF and Wong BA (2002b). Brain manganese concentrations in rats following manganese tetroxide inhalation are unaffected by dietary manganese intake. Neurotoxicology 23(2): 185-95.

Dorman DC, Struve MF, Wong BA, Dye JA and Robertson ID (2006a). Correlation of brain magnetic resonance imaging changes with pallidal manganese concentrations in Rhesus monkeys following subchronic manganese inhalation. Toxicol Sci 92(1): 219-227.

Dorner K, Dziadzka S, Hohn A, Sievers E, Oldigs HD, Schulz-Lell G and Schaub J (1989). Longitudinal manganese and copper balances in young infants and preterm infants fed on breast-milk and adapted cow's milk formulas. Br J Nutr 61(3): 559-72.

Drown DB, Oberg SG and Sharma RP (1986). Pulmonary clearance of soluble and insoluble forms of manganese. J Toxicol Environ Health 17(2-3): 201-12.

Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J and Oberdorster G (2006). Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect 114(8): 1172-8.

Ericson JE, Crinella FM, Clarke-Stewart KA, Allhusen VD, Chan T and Robertson RT (2007). Prenatal manganese levels linked to childhood behavioral disinhibition. Neurotoxicol Teratol 29(2): 181-7.

Erikson KM, Dorman DC, Lash LH and Aschner M (2007a). Manganese inhalation by rhesus monkeys is associated with brain regional changes in biomarkers of neurotoxicity. Toxicol Sci 97(2): 459-66.

Erikson KM, Dorman DC, Lash LH, Dobson AW and Aschner M (2004). Airborne manganese exposure differentially affects end points of oxidative stress in an age- and sex-dependent manner. Biol Trace Elem Res 100(1): 49-62.

Erikson KM, Shihabi ZK, Aschner JL and Aschner M (2002). Manganese accumulates in iron-deficient rat brain regions in a heterogeneous fashion and is associated with neurochemical alterations. Biol Trace Elem Res 87(1-3): 143-56.

Erikson KM, Thompson K, Aschner J and Aschner M (2007b). Manganese neurotoxicity: A focus on the neonate. Pharmacol Ther 113(2): 369-77.

Espinoza A, Rodriguez M, Barragan de la Rosa F and Sabchez J (2001). Size distribution of metals in urban aerosols in Seville (Spain). Atmosph Environ 35(14): 2595-2601.

Fechter LD (1999). Distribution of manganese in development. Neurotoxicology 20(2-3): 197-201.

Fechter LD, Johnson DL and Lynch RA (2002). The relationship of particle size to olfactory nerve uptake of a non-soluble form of manganese into brain. Neurotoxicology 23(2): 177-83.

Fell JM, Reynolds AP, Meadows N, Khan K, Long SG, Quaghebeur G, Taylor WJ and Milla PJ (1996). Manganese toxicity in children receiving long-term parenteral nutrition. Lancet 347(9010): 1218-21.

Ferraz HB, Bertolucci PH, Pereira JS, Lima JG and Andrade LA (1988). Chronic exposure to the fungicide maneb may produce symptoms and signs of CNS manganese intoxication. Neurology 38(4): 550-3.

Fitsanakis VA, Au C, Erikson KM and Aschner M (2006). The effects of manganese on glutamate, dopamine and gamma-aminobutyric acid regulation. Neurochem Int 48(6-7): 426-33.

FNB (2004). Dietary Reference Intake Tables: Elements Table. Food and Nutrition Board, Institutes of Medicine (IOM, 2004). http://www.iom.edu/Object.File/Master/7/294/Webtableminerals.pdf.

Foradori AC, Bertinchamps A, Gulibon JM and Cotzias GC (1967). The discrimination between magnesium and manganese by serum proteins. J Gen Physiol 50(9): 2255-66.

Gavin CE, Gunter KK and Gunter TE (1999). Manganese and calcium transport in mitochondria: implications for manganese toxicity. Neurotoxicology 20(2-3): 445-53.

Gennart JP, Buchet JP, Roels H, Ghyselen P, Ceulemans E and Lauwerys R (1992). Fertility of male workers exposed to cadmium, lead, or manganese. Am J Epidemiol 135(11): 1208-19.

Gianutsos G, Morrow GR and Morris JB (1997). Accumulation of manganese in rat brain following intranasal administration. Fundam Appl Toxicol 37(2): 102-5.

Gibbons RA, Dixon SN, Hallis K, Russell AM, Sansom BF and Symonds HW (1976). Manganese metabolism in cows and goats. Biochim Biophys Acta 444(1): 1-10.

Ginsberg GL, Foos BP and Firestone MP (2005). Review and analysis of inhalation dosimetry methods for application to children's risk assessment. J Toxicol Environ Health A 68(8): 573-615.

Guilarte TR, Chen MK, McGlothan JL, Verina T, Wong DF, Zhou Y, Alexander M, Rohde CA, Syversen T, Decamp E, Koser AJ, Fritz S, Gonczi H, Anderson DW and Schneider JS (2006a). Nigrostriatal dopamine system dysfunction and subtle motor deficits in manganese-exposed non-human primates. Exp Neurol 202(2): 381-90.

Halatek T, Sinczuk-Walczak H and Rydzynski K (2008). Early neurotoxic effects of inhalation exposure to aluminum and/or manganese assessed by serum levels of phospholipid-binding Clara cells protein. J Environ Sci Health A Tox Hazard Subst Environ Eng 43(2): 118-24.

Halatek T, Sinczuk-Walczak H, Szymczak M and Rydzynski K (2005). Neurological and respiratory symptoms in shipyard welders exposed to manganese. International Journal of Occupational Medicine and Environmental Health 18(3): 265-274.

HaMai D, Campbell A and Bondy SC (2001). Modulation of oxidative events by multivalent manganese complexes in brain tissue. Free Radic Biol Med 31(6): 763-8.

Hermans C, Knoops B, Wiedig M, Arsalane K, Toubeau G, Falmagne P and Bernard A (1999). Clara cell protein as a marker of Clara cell damage and bronchoalveolar blood barrier permeability. Eur Respir J 13(5): 1014-21.

Hlavay J, Polyak K, Molnar A and Meszaros E (1998). Determination of the distribution of elements as a function of particle size in aerosol samples by sequential leaching. Analyst 123: 859-863.

HSDB. (2006). *Manganese compounds*. National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~YuJ7Vy:2.

Huang CC, Chu NS, Lu CS, Chen RS and Calne DB (1998). Long-term progression in chronic manganism: ten years of follow-up. Neurology 50(3): 698-700.

Josephs KA, Ahlskog JE, Klos KJ, Kumar N, Fealey RD, Trenerry MR and Cowl CT (2005). Neurologic manifestations in welders with pallidal MRI T1 hyperintensity. Neurology 64(12): 2033-9.

Kafritsa Y, Fell J, Long S, Bynevelt M, Taylor W and Milla P (1998). Long-term outcome of brain manganese deposition in patients on home parenteral nutrition. Arch Dis Child 79(3): 263-5.

Kilburn CJ (1987). Manganese, malformations and motor disorders: findings in a manganese-exposed population. Neurotoxicology 8(3): 421-9.

Komaki H, Maisawa S, Sugai K, Kobayashi Y and Hashimoto T (1999). Tremor and seizures associated with chronic manganese intoxication. Brain Dev 21(2): 122-4.

Kostial K, Blanusa M and Piasek M (2005). Regulation of manganese accumulation in perinatally exposed rat pups. J Appl Toxicol 25(2): 89-93.

Lauwerys R, Roels H, Genet P, Toussaint G, Bouckaert A and De Cooman S (1985). Fertility of male workers exposed to mercury vapor or to manganese dust: a questionnaire study. Am J Ind Med 7(2): 171-6.

Limbach LK, Wick P, Manser P, Grass RN, Bruinink A and Stark WJ (2007). Exposure of engineered nanoparticles to human lung epithelial cells: influence of chemical composition and catalytic activity on oxidative stress. Environ Sci Technol 41(11): 4158-63.

Looker AC, Dallman PR, Carroll MD, Gunter EW and Johnson CL (1997). Prevalence of iron deficiency in the United States. JAMA 277(12): 973-6.

Lucchini R, Apostoli P, Perrone C, Placidi D, Albini E, Migliorati P, Mergler D, Sassine MP, Palmi S and Alessio L (1999). Long-term exposure to "low levels" of manganese oxides and neurofunctional changes in ferroalloy workers. Neurotoxicology 20(2-3): 287-97.

Malecki EA (2001). Manganese toxicity is associated with mitochondrial dysfunction and DNA fragmentation in rat primary striatal neurons. Brain Res Bull 55(2): 225-8.

Martin R (1986). Bioinorganic chemistry of metal ion toxicity. In: Metal ions in biological systems: Concepts on metal ion toxicity. Sigel H. a. S., A. CRC Press. 20: 22-62.

Meco G, Bonifati V, Vanacore N and Fabrizio E (1994). Parkinsonism after chronic exposure to the fungicide maneb (manganese ethylene-bis-dithiocarbamate). Scand J Work Environ Health 20(4): 301-5.

Mena I (1974). The role of manganese in human disease. Ann Clin Lab Sci 4(6): 487-91.

Meral H, Kutukcu Y, Atmaca B, Ozer F and Hamamcioglu K (2007). Parkinsonism caused by chronic usage of intravenous potassium permanganate. Neurologist 13(2): 92-4.

Merck (1976). The Merck Index. Rahway, NJ: Merck and Co.

Mergler D, Baldwin M, Belanger S, Larribe F, Beuter A, Bowler R, Panisset M, Edwards R, de Geoffroy A, Sassine MP and Hudnell K (1999). Manganese neurotoxicity, a continuum of dysfunction: results from a community based study. Neurotoxicology 20(2-3): 327-42.

Mergler D, Huel G, Bowler R, Iregren A, Belanger S, Baldwin M, Tardif R, Smargiassi A and Martin L (1994). Nervous system dysfunction among workers with long-term exposure to manganese. Environ Res 64(2): 151-80.

Miller ST, Cotzias GC and Evert HA (1975). Control of tissue manganese: initial absence and sudden emergence of excretion in the neonatal mouse. Am J Physiol 229(4): 1080-4.

Nagatomo S, Umehara F, Hanada K, Nobuhara Y, Takenaga S, Arimura K and Osame M (1999). Manganese intoxication during total parenteral nutrition: report of two cases and review of the literature. J Neurol Sci 162(1): 102-5.

Newland MC, Cox C, Hamada R, Oberdorster G and Weiss B (1987). The clearance of manganese chloride in the primate. Fundam Appl Toxicol 9(2): 314-28.

NIOSH. (2005). *NIOSH Pocket Guide to Chemical Hazards; Manganese tetroxide (as Mn).* Centers for Disease Control. http://www.cdc.gov/niosh/npg/npgd0381.html

Nong A, Teeguarden JG, Clewell HJ, 3rd, Dorman DC and Andersen ME (2008). Pharmacokinetic modeling of manganese in the rat IV: assessing factors that contribute to brain accumulation during inhalation exposure. J Toxicol Environ Health A 71(7): 413-26.

Normandin L, Ann Beaupre L, Salehi F, St -Pierre A, Kennedy G, Mergler D, Butterworth RF, Philippe S and Zayed J (2004). Manganese distribution in the brain and neurobehavioral changes following inhalation exposure of rats to three chemical forms of manganese. Neurotoxicology 25(3): 433-41.

Normandin L, Carrier G, Gardiner PF, Kennedy G, Hazell AS, Mergler D, Butterworth RF, Philippe S and Zayed J (2002). Assessment of bioaccumulation, neuropathology, and neurobehavior following subchronic (90 Days) inhalation in Sprague-Dawley rats exposed to manganese phosphate. Toxicol. Appl. Pharmacol. 183(2): 135-145.

NWU. (2006). Nutrition Fact Sheet: Manganese. Retrieved 8-17-07, from http://www.feinberg.northwestern.edu/nutrition/factsheets/manganese.html.

OEHHA. (2000). *The Air Toxics Hot Spots Program Risk Assessment Guidelines Part IV: Technical Support Document for Exposure Assessment and Stochastic Analysis*. Air Toxicology and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

Ono J, Harada K, Kodaka R, Sakurai K, Tajiri H, Takagi Y, Nagai T, Harada T, Nihei A, Okada A and et al. (1995). Manganese deposition in the brain during long-term total parenteral nutrition. JPEN J Parenter Enteral Nutr 19(4): 310-2.

Pellizzari ED, Clayton CA, Rodes CE, Mason RE, Piper LL, Fort B, Pfeifer G and Lynam D (2001). Particulate matter and manganese exposures in Indianapolis, Indiana. Journal Expo Anal Environ Epidemiol 11(6): 423-40.

Pine M, Lee B, Dearth R, Hiney JK and Dees WL (2005). Manganese acts centrally to stimulate luteinizing hormone secretion: a potential influence on female pubertal development. Toxicol Sci 85(2): 880-5.

Ponnapakkam TP, Bailey KS, Graves KA and Iszard MB (2003). Assessment of male reproductive system in the CD-1 mice following oral manganese exposure. Reprod Toxicol 17(5): 547-51.

Reaney SH, Bench G and Smith DR (2006). Brain accumulation and toxicity of Mn(II) and Mn(III) exposures. Toxicol Sci 93(1): 114-124.

Rice TM, Clarke RW, Godleski JJ, Al-Mutairi E, Jiang NF, Hauser R and Paulauskis JD (2001). Differential ability of transition metals to induce pulmonary inflammation. Toxicol Appl Pharmacol 177(1): 46-53.

Roels H, Lauwerys R, Buchet JP, Bernard A, Barthels A, Oversteyns M and Gaussin J (1982). Comparison of renal function and psychomotor performance in workers exposed to elemental mercury. Int Arch Occup Environ Health 50(1): 77-93.

Roels H, Meiers G, Delos M, Ortega I, Lauwerys R, Buchet JP and Lison D (1997). Influence of the route of administration and the chemical form (MnCl2, MnO2) on the absorption and cerebral distribution of manganese in rats. Arch Toxicol 71(4): 223-30.

Roels HA, Eslava MIO, Ceulemans E, Robert A and Lison D (1999). Prospective study on the reversibility of neurobehavioral effects in workers exposed to manganese dioxide. Neurotoxicology 20: 255-272.

Roels HA, Ghyselen P, Buchet JP, Ceulemans E and Lauwerys RR (1992). Assessment of the permissible exposure level to manganese in workers exposed to manganese dioxide dust. Br J Ind Med 49(1): 25-34.

Rose C, Butterworth RF, Zayed J, Normandin L, Todd K, Michalak A, Spahr L, Huet PM and Pomier-Layrargues G (1999). Manganese deposition in basal ganglia structures results from both portal-systemic shunting and liver dysfunction. Gastroenterology 117(3): 640-4.

Sadek AH, Rauch R and Schulz Paul E. (2003). Parkinsonism due to manganism in a welder. Department of Neurology, Baylor College of Medicine, Houston, Texas 77030, USA. United States

Salehi F, Krewski D, Mergler D, Normandin L, Kennedy G, Philippe S and Zayed J (2003). Bioaccumulation and locomotor effects of manganese phosphate/sulfate mixture in Sprague-Dawley rats following subchronic (90 days) inhalation exposure. Toxicol. Appl. Pharmacol. 191(3): 264-271.

Schneider JS, Decamp E, Koser AJ, Fritz S, Gonczi H, Syversen T and Guilarte TR (2006). Effects of chronic manganese exposure on cognitive and motor functioning in non-human primates. Brain Res 1118(1): 222-31.

Schroeder HA, Balassa JJ and Tipton IH (1966). Essential trace metals in man: manganese. A study in homeostasis. J Chronic Dis 19(5): 545-71.

Shen XM and Dryhurst G (1998). Iron- and manganese-catalyzed autoxidation of dopamine in the presence of L-cysteine: possible insights into iron- and manganese-mediated dopaminergic neurotoxicity. Chem Res Toxicol 11(7): 824-37.

Shiotsuka R. (1984). Inhalation toxicity of manganese dioxide and magnesium oxide-manganese dioxide mixture. NTIS ADA-148868. Inhalation Toxicology Facility, Medical Dept., Brookhaven National Laboratory, Uptown, NY. Fort Detrick, MD

Shukakidze A, Lazriev I and Mitagvariya N (2003). Behavioral impairments in acute and chronic manganese poisoning in white rats. Neurosci Behav Physiol 33(3): 263-7.

Shukla GS and Chandra SV (1977). Levels of sulfhydryls and sulfhydryl-containing enzymes in brain, liver and testis of manganese treated rats. Arch Toxicol 37(4): 319-25.

Sikk K, Taba P, Haldre S, Bergquist J, Nyholm D, Zjablov G, Asser T and Aquilonius SM (2007). Irreversible motor impairment in young addicts--ephedrone, manganism or both? Acta Neurol Scand 115(6): 385-9.

Singh M, Jaques P and Sioutas C (2002). Size distribution and diurnal characteristics of particle-bound metals in source and receptor sites of the Los Angeles basin. Atmosph Environ 36(10): 1675-1689.

Struve MF, McManus BE, Wong BA and Dorman DC (2007). Basal ganglia neurotransmitter concentrations in rhesus monkeys following subchronic manganese sulfate inhalation. Am J Ind Med 50(10): 772-8.

Takser L, Mergler D, Hellier G, Sahuquillo J and Huel G (2003). Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology (Amsterdam) 24(4-5): 667-674.

Tapin D, Kennedy G, Lambert J and Zayed J (2005). Bioaccumulation and locomotor effects of manganese sulfate in Sprague-Dawley rats following subchronic (90 days) inhalation exposure. Toxicol Appl Pharmacol 26(3): 351-9.

Teeguarden JG, Dorman DC, Covington TR, Clewell HJ, 3rd and Andersen ME (2007a). Pharmacokinetic modeling of manganese. I. Dose dependencies of uptake and elimination. J Toxicol Environ Health A 70(18): 1493-504.

Teeguarden JG, Dorman DC, Nong A, Covington TR, Clewell HJ, 3rd and Andersen ME (2007b). Pharmacokinetic modeling of manganese. II. Hepatic processing after ingestion and inhalation. J Toxicol Environ Health A 70(18): 1505-14.

Teeguarden JG, Gearhart J, Clewell HJ, 3rd, Covington TR, Nong A and Andersen ME (2007c). Pharmacokinetic modeling of manganese. III. Physiological approaches accounting for background and tracer kinetics. J Toxicol Environ Health A 70(18): 1515-26.

Ter Haar GL, Griffing ME, Brandt M, Oberding DG and Kapron M (1975). Methylcyclopentadienyl manganese tricarbonyl as an antiknock: composition and fate of manganese exhaust products. J. Air Pollut. Control Assoc. 25(8): 858-860.

Thiruchelvam M, Richfield EK, Baggs RB, Tank AW and Cory-Slechta DA (2000). The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: implications for Parkinson's disease. J Neurosci 20(24): 9207-14.

Thompson K, Molina RM, Donaghey T, Schwob JE, Brain JD and Wessling-Resnick M (2007). Olfactory uptake of manganese requires DMT1 and is enhanced by anemia. FASEB J 21(1): 223-30.

Tran TT, Chowanadisai W, Crinella FM, Chicz-DeMet A and Lonnerdal B (2002a). Effect of high dietary manganese intake of neonatal rats on tissue mineral accumulation, striatal dopamine levels, and neurodevelopmental status. Neurotoxicology 23(4-5): 635-43.

Tran TT, Chowanadisai W, Lonnerdal B, Le L, Parker M, Chicz-Demet A and Crinella FM (2002b). Effects of neonatal dietary manganese exposure on brain dopamine levels and neurocognitive functions. Neurotoxicology 23(4-5): 645-51.

U.S.EPA. (1993). Manganese (CASRN 7439-96-5) Reference concentration for chronic inhalation exposure (RfC). http://www.epa.gov/IRIS/subst/0373.htm Retrieved 3/28/06, from http://www.epa.gov/IRIS/subst/0373.htm.

Valois AA and Webster WS (1989). Retention and distribution of manganese in the mouse brain following acute exposure on postnatal day 0, 7, 14 or 42: an autoradiographic and gamma counting study. Toxicology 57(3): 315-28.

Vitarella D, Wong BA, Moss OR and Dorman DC (2000). Pharmacokinetics of inhaled manganese phosphate in male Sprague-Dawley rats following subacute (14-day) exposure. Toxicol Appl Pharmacol 163(3): 279-85.

Wang XF and Cynader MS (2001). Pyruvate released by astrocytes protects neurons from copper-catalyzed cysteine neurotoxicity. J Neurosci 21(10): 3322-31.

Wariishi H, Valli K, Renganathan V and Gold MH (1989). Thiol-mediated oxidation of nonphenolic lignin model compounds by manganese peroxidase of Phanerochaete chrysosporium. J Biol Chem 264(24): 14185-91.

Wennberg A, Hagman M and Johansson L (1992). Preclinical neurophysiological signs of parkinsonism in occupational manganese exposure. Neurotoxicology 13(1): 271-4.

Wittczak T, Dudek W, Krakowiak A, Walusiak J and Palczynski C (2008). Occupational asthma due to manganese exposure: a case report. Int J Occup Med Environ Health 21(1): 81-3.

Woolf A, Wright R, Amarasiriwardena C and Bellinger D (2002). A child with chronic manganese exposure from drinking water. Environ Health Perspect 110(6): 613-616.

Zhang G, Liu D and He P (1995). [Effects of manganese on learning abilities in school children]. Zhonghua Yu Fang Yi Xue Za Zhi 29(3): 156-8.

Mercury Reference Exposure Levels

(Hg^0 Elemental; Quicksilver)

CAS 7439-97-6

1. Summary

Elemental mercury exposures adversely affect several organ systems. The effects of acute, high level inhalation exposures first appear in the lungs as pulmonary dysfunction, possibly followed by respiratory failure leading to death. At lower levels of exposure, the kidneys and brain, especially the developing brain, are more sensitive targets. Short term maternal exposure to mercury vapor during pregnancy may result in long lasting neurobehavioral effects in the offspring, an effect upon which the acute REL is based. Chronic, low level exposures also adversely affect the central nervous system and manifest as motor deficits (tremors, unsteady gait, performance decrements), mood changes (irritability, nervousness), poor concentration, short-term memory deficits, tremulous speech, blurred vision, paresthesia, and decreased nerve conduction. Renal and cardiovascular functions are also impaired with long term exposure. This REL focuses on inhalation exposures. There is a large body of literature on methylmercury poisoning as well as the toxicology of ingested mercury. Much of the latter is reviewed in OEHHA's documentation of the Public Health Goal for drinking water (OEHHA, 1999)

1.1 Mercury Acute REL

Reference Exposure Level	**0.6 μg Hg/m³ (0.07 ppb Hg^0)**
Critical effect(s)	CNS disturbances in offspring
Hazard Index target(s)	Nervous system

1.2 Mercury 8-Hour REL

Reference Exposure Level	**0.06 μg Hg/m³ (0.007 ppb Hg^0)**
Critical effect(s)	Impairment of neurobehavioral functions in humans
Hazard Index target(s)	Nervous system

1.3 Mercury Chronic REL

Reference Exposure Level	**0.03 μg Hg/m³ (0.004 ppb Hg^0)**
Critical effect(s)	Impairment of neurobehavioral functions in humans
Hazard Index target(s)	Nervous system

2. Physical & Chemical Properties - Elemental Mercury

Description	Silver-white, heavy, mobile, odorless, liquid metal
Molecular formula	Hg^0
Molecular weight	200.59 g/mol
Density	13.53 g/cm^3 @ 25° C
Boiling point	356.73° C
Melting point	-38.7° C
Vapor pressure	2 x 10^{-3} mm Hg @ 25° C
Solubility	soluble in nitric acid, to some extent in lipids, and up to 0.28 µmol in water @ 25° C
Odor threshold	odorless
Conversion factor	1 ppm in air = 8.34 mg/m^3 @ 25° C

3. Occurrence and Major Uses

Mercury and mercury-containing compounds are widely used in diverse applications. Thermometers, barometers and thermostats take advantage of mercury's uniform temperature-dependent volume expansion over a broad temperature range. It is used in mercury arc and fluorescent lamps, as a catalyst in oxidation of organic compounds, in the extraction of gold and silver from ores, and as a cathode in electrolysis. It is also used in pulp and paper manufacturing, as a component of batteries, in dental amalgams, and in the manufacture of switching devices such as oscillators, the manufacture of chlorine and caustic soda, as a lubricant, and as a laboratory reagent. To a lesser extent mercury has been used as a grain fumigant, in pharmaceuticals, agricultural chemicals, and as a preservative (ACGIH, 1986).

The annual statewide emissions of mercury from mobile, stationary and natural sources reported in the California Toxics Inventory for 2004 were estimated to be 18 tons (CARB, 2005a). Statewide ambient levels of mercury in 2002 were 1.7 ng/m^3 (CARB, 2005b). Mercury emitted in the metallic form is slowly oxidized in the atmosphere to the ionic mercurous and mercuric (+1 and +2) forms, which are much more soluble in water. These forms dissolve in raindrops and are deposited onto land and water. Much of this precipitation enters sediment of streams or other water bodies, where it is converted to methylmercury and can be accumulated by fish. Thus human exposure to air-borne mercury may be direct, via inhalation, and indirect, through a diet containing contaminated fish. For the purposes of evaluating a Reference Exposure Level, however, we focus on studies of inhalation exposure to mercury.

4. Metabolism / Toxicokinetics

Inhalation exposure to mercury is usually to vapors of the elemental form. However, combustion processes may also emit mercury salts (chlorides and oxides). Thus inhalation exposure to these forms also occurs. Exposure to the inorganic forms of mercury, the mercurous and mercuric salts, also occurs via the oral route. However, absorption from the intestinal tract is much less efficient (2-38%) than from the lungs (70-80%) (ATSDR, 1999). To protect against oral exposure to inorganic mercury via drinking water, OEHHA (1999) has developed a public health goal (PHG) of 0.0012 mg/L (1.2 ppb) as a level of exposure expected to pose no significant

health risk to individuals consuming the water on a daily basis. The difference between the PHG and the REL values reported in this document in part reflects differences in the toxicokinetics by the different routes of exposure. For inhalation exposure to mercury vapor, modeling based on human and experimental animal studies suggests that approximately 80% of inhaled mercury is deposited in the respiratory tract, of which about 70% is rapidly absorbed into the blood with a half-time of around 1 min. The remainder is absorbed more slowly with half-times of 8 hr to 5 days (Leggett et al., 2001). Absorption is markedly decreased if the breathing is done only through the mouth (Teisinger and Fiserova-Bergerova, 1965). It is not clear whether this difference is related to the direct uptake of mercury from nasal passages but mercury is known to be transported via olfactory nerves directly to the brain (Tjalve and Henriksson, 1999). In the blood, elemental mercury (Hg^0) may be oxidized by catalase and peroxidase to the more toxic inorganic forms. Cellular membranes and the blood-brain barrier are readily permeable to Hg^0, but much less so to the inorganic forms. Residual Hg^0 in the blood may enter target cells and be oxidized to the mercuric form intracellularly, effectively trapping it in the cells. The biological half-life of mercury in the human head is reported to be 21 days, and 64 days in the kidney (Hursh et al., 1976). Mercury is eliminated in urine, feces and exhaled air.

Mercury exerts its toxicity through several mechanisms mainly related to the high affinity of the mercuric ion for sulfhydryl groups. By binding to non-protein sulfhydryls such as glutathione and N-acetyl cysteine, mercury alters intracellular thiol status, thus promoting oxidative stress and lipid peroxidation. Mercury interacts with the mitochondrial electron transport chain resulting in increased H_2O_2. There is a concomitant depletion of mitochondrial glutathione, depolarization of the inner mitochondrial membrane, and increased susceptibility of the mitochondrial membrane to peroxidation. Mitochondrial function is thus impaired and oxidative stress increased (Lund et al., 1993). In addition to mercury's pro-oxidant effects, the binding of mercury by sulfhydryl-containing proteins disrupts a broad range of critical cellular functions such as microtubule polymerization (Yole et al., 2007), DNA transcription (Rodgers et al., 2001), glutamine synthesis (Allen et al., 2001), and calcium homeostasis (Yole et al., 2007). These effects may lead to cell dysfunction and death, an effect that is exacerbated by mercury's ability to promote auto-immune responses (Rowley and Monestier, 2005). Indeed, among genetically susceptible individuals, much of the renal pathology associated with mercury exposure has been attributed to auto-antibodies to renal proteins (Hua et al., 1993). Disruption of cellular processes during development can have severe and long-lasting effects. This is especially true during the growth and organization of the central nervous system as it is critically dependent on cell division and neuronal migration. These processes in turn depend on microtubule polymerization which is powerfully inhibited by both the mercuric ion and methylmercury.

5. Acute Toxicity of Mercury

5.1 Acute Toxicity to Adult Humans

The respiratory tract is the first organ system affected in cases of acute inhalation poisoning (Levin et al., 1988). Acute exposure to Hg^0 can lead to shortness of breath within 24 hours and a rapidly deteriorating course leading to death due to respiratory failure (Kanluen and Gottlieb, 1991; Asano et al., 2000). In a case report, Kanluen and Gottlieb (1991) observed four individuals from a private home where silver was being smelted from dental amalgam containing an unknown amount of Hg^0. All individuals died 9-23 days post-exposure from respiratory distress despite treatment with dimercaprol, a mercury chelator. Autopsy revealed acute lung injury characterized by necrotizing bronchiolitis with edema, emphysema, and obliteration of alveolar spaces with extensive interstitial fibrosis. The concentrations of mercury to which the individuals were exposed and the duration of exposure are not known.

Central nervous system (CNS) effects such as tremors or increased excitability are sometimes seen in cases of acute accidental exposures (Netterstrom et al., 1996). Long-term effects from a single exposure to Hg^0 were reported in 6 male workers exposed to an estimated concentration of 44 mg Hg/m^3 for a period of several hours (McFarland and Reigel, 1978). Long-term CNS effects included nervousness, irritability, lack of ambition, and loss of sexual drive for several years. Shortness of breath also persisted for years in all cases. Acute inhalation exposure to Hg^0 vapors from broken thermometers resulted in generalized skin eruptions in 15 individuals (Nakayama et al., 1983). The doses and durations of exposure were not estimated.

A similar symptomatology was reported by Sexton et al. (1978) following the spillage of 100-300 ml of elemental mercury in two mobile homes that exposed 11 people to mercury vapor for one to two months. Following one to two weeks of exposure, the most intensely exposed residents, three teenage girls, reported the onset of anorexia, painful mouth, abdominal cramps, mild diarrhea, bleeding gingiva, irritated eyes, insomnia, difficulty concentrating and general restlessness. Prior to the girls' hospitalization, changes in academic performance, handwriting and personality were noted by the girls' teachers. A similar constellation of symptoms including intention tremor was subsequently observed in the other eight exposed residents. Skin rashes of varying severity were also seen among five of the residents. Blood mercury levels ranged from 183 to 620 ng/ml (normal is < 5 ng/ml). The highest air mercury level measured in one of the vacated and sealed trailers was 1.0 mg/m^3 five months after the initial spill. Neurological exams at two to four months following termination of exposure were normal for eight of the residents. However, at four months, two of the intensely exposed girls still showed neurological abnormalities as manifested in difficulties copying simple diagrams, and abnormal electroencephalograms.

The acute effects of inhalation exposure to mercury may be compounded by simultaneous dietary intake of methylmercury. The use of mercury amalgamation in the recovery of gold in the Amazon Basin has resulted in locally elevated mercury levels both in indoor air in gold shops (250-40,600 ng/m^3), and in ambient urban air (20-5,800 ng/m^3) (Hacon et al., 1995), thus increasing the opportunities for both acute and long-term exposures. At the same time, gold extraction activities have caused mercury contamination of waterways resulting in a concomitant increase in methylmercury in the diet from the consumption of contaminated fish (Cordier et al.,

1998). Adverse neurological and otological effects have been associated with elevated blood mercury levels in both adults and children in this environment (Counter et al., 1998).

Predisposing Conditions for Mercury Toxicity

Medical: Persons with preexisting nervous system disorders or kidney diseases might be at increased risk of mercury toxicity. Also at higher risk are persons previously sensitized to mercury (Lerch and Bircher, 2004), and those with genetic susceptibililty to mercury-induced hypersensitivity (Warfvinge et al., 1995). Developing organisms (fetuses and infants) are especially susceptible to the neurotoxicity of mercury (USEPA, 1997).

Other: People who consume significant amounts of fish from areas with advisories for daily fish intake due to mercury contamination may be more susceptible to the chronic toxicity of airborne mercury due to existing body burden.

5.2 Acute Toxicity to Infants and Children

The data regarding the toxic effects of acute exposure of children to Hg^0 are largely limited to case reports with little or no information on actual exposure levels. In children who inhale high levels of toxic Hg^0 vapors, pulmonary dysfunction is the primary cause of mortality. For example, autopsy of a 4-month-old child who died following acute exposure to Hg^0 vapors revealed pulmonary and general edema, nephrotic degeneration, ventricular dilation, and a greyish, necrotic appearance in the digestive mucosa (Campbell, 1948). In another case study, severe interstitial pneumonitis, erosion of the bronchial epithelium, membrane lesions of the alveoli and alveolar ducts, and significantly elevated Hg in the kidneys and liver were documented by Matthes et al. (1958) following the deaths of three children aged 4, 20, and 30 months from acute Hg^0 vapor exposure in the home. Cases of CNS disturbances, including irritability, insomnia, malaise, anorexia, fatigue, ataxia, and headache have been reported in children exposed to vapor from spilled elemental mercury in their homes (Florentine and Sanfilippo, 1991).

5.3 Acute Toxicity to Experimental Animals

As reported for humans, acute inhalation exposure of experimental animals to high levels of mercury is associated with pulmonary toxicity. However, the effects of mercury inhalation following short term exposure have also been examined in the context of neurotoxicity, notably neurobehavioral effects, and mercury deposition and distribution in the nervous system, as well as pathological changes in various organs.

Pathological changes in lung tissues similar to those reported in humans (edema, fibrosis, and necrosis of alveolar epithelium and hyaline membranes) were observed by Livardjani et al. (1991) in rats exposed to 26 mg (3.1 ppm) Hg/m^3 for 1 hour, or 27 mg (3.2 ppm) for 2 hours. A dose-dependence of lung pathology and mortality was reported. No mortality was observed during the subsequent 15 days following the 1 hour exposure, while 50% mortality and more severe lesions were seen during the first 5 days following the 2 hour exposure.

In a study of pulmonary effects of mercury inhalation, as well as the possible role of metallothionein (MT), Yoshida et al. (1999) exposed both MT-null and wild-type mice to 6.6 - 7.5 mg/m^3 (0.79 - 0.90 ppm) mercury vapor for 4 hours on 3 consecutive days. Examination of the lungs 24 hours after exposure revealed severe congestion, atelectasis (incomplete expansion of the lung), and mild hemorrhage of the alveoli in MT-null mice, along with 60% mortality. Among wild-type mice, these pulmonary effects were much less severe, pulmonary MT expression was markedly increased, and no lethality was observed. Mercury was found bound to MT in the lungs of wild-type, but not in MT-null mice. MT thus appears to ameliorate the effects of mercury inhalation.

The neurobehavioral manifestations in the offspring of mice with maternal exposure to mercury vapor during pregnancy suggest damage to motor control and learning centers. In the study upon which the acute REL derivation is based, Danielsson et al. (1993) exposed pregnant rats (12 per group) by inhalation to 1.8 mg/m³ (0.22 ppm) of Hg^0 vapor for 1 hour/day (0.07 mg/kg/d) or 3 hours/day (0.2 mg/kg/d) during gestational days 11-14 and 17-20. The dose level was selected to avoid maternal toxicity. Tests of motor activity (locomotion, rearing, rearing time, total activity) in the offspring at 3 months of age revealed significant dose-dependent deficits compared to controls ($p < 0.01$). When tested at 14 months of age, the hypoactivity seen at 3 months was no longer apparent and, in the 0.2 mg/kg/d dose group, was replaced with significant hyperactivity (Table 5.3.1).

TABLE 5.3.2 EFFECTS OF PRENATAL METALLIC MERCURY ON MOTOR ACTIVITY

		3 months			14 months		
		Control	**0.07**	**0.2**	**Control**	**0.07**	**0.2**
Activity	**Day**	(SEM)	mg/kg/d	mg/kg/d	(SEM)	mg/kg/d	mg/kg/d
Locomotion	1	2785 (135)	2141 (104)*	2212 (135)*	1862 (119)	1289 (167)	1767 (127)
	2	2069 (127)	1432 (119)*	1385 (143)*	1194 (111)	1218 (104)	1512 (119)
	3	1719 (175)	1663 (191)	1090 (135)*	1162 (111)	915 (135)	1369 (119)
Rearing	1	404 (25)	321 (25)*	338 (25)*	204 (22)	143 (20)	210 (27)
	2	312 (29)	190 (20)*	161 (25)*	87 (22)	110 (28)	123 (22)
	3	247 (29)	238 (18)	157 (32)*	84 (18)	98 (25)	106 (18)
Rearing time	1	431 (19)	243 (20)*	232 (22)*	159 (21)	78 (24)	167 (26)
	2	269 (21)	138 (23)*	160 (24)*	66 (19)	99 (24)	114 (23)
	3	212 (21)	179 (23)	138 (21)*	87 (17)	76 (22)	138 (24)
Total activity	1	4854 (271)	3836 (318)*	3979 (302)*	3565 (302)	2435 (223)*	3151 (271)
	2	3804 (223)	2737 (239)*	2817 (350)*	2308 (255)	2324 (302)	3151 (334)*
	3	3183 (318)	3183 (350)	2132 (318)*	2228 (255)	2069 (271)	2546 (2711)

*p<0.01 Data estimated from Danielsson et al. (1993) Figure 1.

Significant learning deficits (swim maze performance) were observed in the 0.2 mg/kg/d-exposed, but not the lower-exposure rats tested at 15 months of age ($p < 0.05$) (Table 5.3.2). The brain concentrations of mercury in the 0.2 mg/kg/d dose group (0.012 mg/kg) were 2.5-fold higher than in the 0.07 mg/kg/d dose group (0.005 mg/kg), and 12-fold higher than in the control group (0.001 mg/kg).

TABLE 5.3.3 PRENATAL METALLIC MERCURY AND LEARNING DEFICITS

		7 months			15 months	
	Control	0.07	0.2	Control	0.07	0.2
Morris maze	**Day**	mg/kg/d	mg/kg/d		mg/kg/d	mg/kg/d
	1	53	48	46	42	40	29
	2	30	41	26	29	21	13*

*p<0.01 Data estimated from Danielsson et al. (1993) Figure 3.

These data indicate adverse effects of mercury exposure on the developing brain, but it is not clear at what nervous tissue levels effects first manifest.

To evaluate mercury deposition in neurons at low exposure levels, Pamphlett and Coote (1998) exposed female BALB/c mice to mercury vapor at 25 µg/m^3 (0.003 ppm) for 2-20 hr, or to 500 µg/m^3 (0.06 ppm) for 5-240 min. At 25 µg/m^3, mercury was first found in the perikarya of scattered large motor neurons in the lateral anterior horn of the spinal cord after 12 hr of exposure. Exposure at this level for 16 and 20 hr resulted in labeling of most of the large neurons of this area. By comparison, mercury was found in renal tubular epithelium after only 2 hr of exposure. Mice that survived longer than 6 weeks showed no mercury in the renal epithelia while mercury persisted in the brainstem motor neurons up to 30 weeks. At the higher dose of 500 µg/m^3, mercury labeling of spinal motor neurons was seen after only 30 min. The doses that resulted in mercury uptake into mouse motor neurons in these experiments are similar to those that workers in mercury-using occupations may receive in the course of a few hours. While the toxicological significance of the observed mercury labeling was not addressed in these mice, the accumulation of mercury in the motor neurons is consistent with the behavioral alterations reported above.

The effects of short term, high level exposure to mercury are not limited to pulmonary and nervous tissues. Severe cellular degeneration and necrosis were observed in the kidneys, brain, colon, and heart tissue of 2 rabbits exposed for 4 hours to 29.7 mg Hg/m³ (3.6 ppm) (Ashe et al., 1953). Exposure of rabbits to 31.3 mg Hg/m³ (3.8 ppm) for 1 hour resulted in moderate pathological changes (unspecified), but no necrosis, in the brain and kidney. In contrast, heart and lung tissues showed mild pathologic changes (Ashe et al., 1953). Increased duration (6 hours/day for 5 days) of exposure at this concentration was lethal.

6. Chronic Toxicity of Mercury

6.1 Chronic Toxicity to Adult Humans

This section briefly summarizes a large body of literature on mercury toxicity, emphasizing studies of inhalation exposure useful in the development of the 8-hr and chronic reference exposure levels. The reader is referred to OEHHA (1999) for more information on measuring toxicity by the oral route of exposure. The effects of chronic exposure to mercury vapor have been known for centuries and are most pronounced in the central nervous system. Toxic effects include tremors (mild or severe), unsteady gait, irritability, poor concentration, short-term memory deficits, tremulous speech, blurred vision, performance decrements, paresthesia, and decreased nerve conduction (Smith et al., 1970; Langolf et al., 1978; Fawer et al., 1983; Piikivi et al., 1984; Albers et al., 1988; Kishi et al., 1993). While some motor system disturbances can be reversed upon cessation of exposure, memory deficits may be permanent (Kishi et al., 1993). Studies have shown effects such as tremor and decreased cognitive skills in workers exposed to approximately 25 $\mu g/m^3$ (0.003 ppm) mercury vapor (Piikivi et al., 1984; Piikivi and Hanninen, 1989; Piikivi and Toulonen, 1989) (see discussion below).

The kidney is also a sensitive target organ of mercury toxicity. Effects such as proteinuria, proximal tubular and glomerular changes, albuminuria, glomerulosclerosis, and increased urinary N-acetyl-β-glucosaminidase have been seen in workers exposed to approximately 25-60 $\mu g/m^3$ (0.003 - 0.007 ppm) mercury vapor (Roels et al., 1982; Bernard et al., 1987; Barregard et al., 1988; Piikivi and Ruokonen, 1989).

Chronic exposure to mercury vapors has also resulted in cardiovascular effects such as increased heart rate and blood pressure (Piikivi, 1989; Fagala and Wigg, 1992; Taueg et al., 1992), and in leukocytosis and neutrophilia (Fagala and Wigg, 1992).

A number of other studies with similar exposure levels also found adverse psychological and neurological effects in exposed versus unexposed individuals. Fawer et al. (1983) measured intention tremor with an accelerometer attached to the third metacarpal of the right hand in 26 male workers (mean age of 44 years) exposed to low concentrations of mercury vapor. The men worked either in a chloralkali plant (n = 12), a fluorescent tube manufacturing plant (n = 7), or in acetaldehyde production (n = 7). Twenty-five control subjects came from different parts of the same plants and were not occupationally exposed to mercury. The average exposure as measured by personal air sampling was 0.026 mg/m^3 (0.003 ppm) and the average duration of exposure was 15 years. The measurements of intention tremor were significantly higher in exposed workers than in controls ($p = 0.011$). Using the average exposure as a LOAEL and adjusting for occupational ventilation rates and workweek, the resultant LOAEL is 0.009 mg/m^3 (0.001 ppm).

Piikivi and Tolonen (1989) studied the effects of long-term exposure to mercury vapor on electroencephalograms (EEGs) of 41 chloralkali workers exposed for a mean of 15.6 years as compared to 41 matched controls. EEGs were analyzed both qualitatively and quantitatively. In the qualitative analysis, EEGs were interpreted visually with classification of normality and abnormality based on a previously established scale that separated focal, generalized and paroxysmal disturbances into four classes (normal, or mildly, moderately, or severely disturbed).

Exposed workers, who had blood mercury levels of 11.6 μg/L, tended to have an increased number of EEG abnormalities and brain activity was found to be significantly lower than matched controls ($p < 0.001$). The abnormalities were most prominent in the parietal cortex, but absent in the frontal cortex. The authors used a conversion factor calculated by Roels et al. (1989) to extrapolate from blood mercury levels of 12 μg/L to an air concentration of 25 μg/m^3 (0.003 ppm).

Another study by Piikivi (1989) examined subjective and objective symptoms of autonomic dysfunction in the same 41 chloralkali workers described above. The exposed workers had mean blood levels of 11.6 μg/L corresponding to a TWA exposure of 25 μg Hg/m^3 in air (Roels et al., 1987). The workers were tested for pulse rate variation in normal and deep breathing, the Valsalva maneuver, vertical tilt, and blood pressure responses during standing and isometric work. The only significant difference in subjective symptoms was an increased reporting of palpitations in exposed workers. The objective tests demonstrated an increase in pulse rate variations at 30 μg Hg/m^3 (0.006 ppm; extrapolated from blood levels based on methods of Roels et al. (1987)), which is indicative of autonomic reflex dysfunction.

Piikivi and Hanninen (1989) studied subjective symptoms and psychological performance on a computer-administered test battery in 60 chloralkali workers exposed to approximately 25 μg/m^3 mercury vapor for a mean of 13.7 years. The subjective symptoms, evaluated by questionnaire, included the frequency or intensity of memory disturbances, difficulties concentrating, sleep disorders, and hand tremors. In addition a mood scale was used to evaluate tension, depression, anger, fatigue, and confusion. The psychomotor tests included finger tapping, eye-hand coordination, symbol digit substitution, pattern comparison, and a continuous performance test. Memory and learning effects were captured on tests of associate learning, associate memory, pattern memory, and serial digit learning. A statistically significant increase in subjective symptoms of sleep disturbance and memory disturbance was noted in the exposed workers ($p < 0.001$), as were increased anger, fatigue and confusion ($p < 0.01$). There were no differences in objective measures of memory, learning, or motor abilities, with the exception of poorer eye-hand coordination ($p < 0.001$).

A study by Ngim et al. (1992) assessed neurobehavioral performance in a cross-sectional study of 98 dentists exposed to a TWA concentration of 14 μg Hg/m^3 (range 0.7 to 42 μg/m^3) compared to 54 controls with no history of occupational exposure to mercury. Exposed dentists were matched to the control group for age, amount of fish consumption, and number of amalgam fillings. Air concentrations were measured with personal sampling badges over typical working hours (8-10 hours/day) and converted to a TWA. Blood samples were also taken (average 9.8 μg/L). The average concentration in air was estimated at 23 μg Hg/m^3 when the methods of Roels et al. (1987) were used. The average duration in this study of dentists was only 5.5 years, shorter than the above studies. The performance of the dentists was significantly worse than controls on a number of neurobehavioral tests measuring motor speed (finger tapping), visual scanning, visuomotor coordination and concentration, visual memory, and visuomotor coordination speed ($p < 0.05$). These neurobehavioral changes are consistent with central and peripheral neurotoxicity commonly observed in cases of chronic mercury toxicity.

Liang et al. (1993) investigated workers in a fluorescent lamp factory with a computer-administered neurobehavioral evaluation system and a mood-inventory profile. The cohort

consisted of 88 individuals (19 females and 69 males) exposed for at least 2 years prior to the study. Exposure was monitored with area samplers and ranged from 8 to 85 μg Hg/m^3 across worksites. The average level of exposure was estimated at 33 μg Hg/m^3 and the average duration of exposure was estimated at 15.8 years. The exposed cohort performed significantly worse than the controls on tests of finger tapping, mental arithmetic, two digit searches, switching attention, and visual reaction time ($p < 0.05$-0.01). The effects on performance persisted after controlling for chronological age as a confounding factor.

6.2 Chronic Toxicity to Infants and Children

A number of case studies indicate that long-term exposure to Hg^0 in children is associated with severe arterial hypertension, acrodynia, seizures, tachycardia, anxiety, irritability and general malaise (Sexton et al., 1978; Torres et al., 2000). These symptoms are consistent with the brain and kidneys as the principal target organs for Hg^0. By comparison, for methylmercury (MeHg), the brain is the most toxicologically relevant organ. An extensive literature supports the association between chronic MeHg exposure and neurological and developmental deficits in children (Choi, 1989; Harada, 1995; Grandjean et al., 1999). Unlike inorganic mercury, both Hg^0 and MeHg easily cross cell membranes, the blood brain barrier, and the placenta (Ask et al., 2002). Intracellular oxidation of Hg^0 and the slower demethylation of MeHg both lead to the mercuric ion that binds cellular macromolecules, trapping it within the cell and contributing to the toxicity associated with exposures to the respective forms. While the complete mechanisms of toxicity for the two forms are not well understood and are likely not identical, there are important similarities. Methylmercury and the mercuric ion formed from Hg^0 avidly bind to protein sulfhydryls and may inactivate enzymes. Disruption of protein synthesis has been reported after exposure to either Hg^0 or MeHg, although the former is the more powerful inhibitor (NAS, 2000). The neurotoxic effects observed in adult rats following *in utero* exposure to Hg^0, MeHg, or both, are reportedly similar with MeHg potentiating the effects of Hg^0 (Fredriksson et al., 1996). Given the high susceptibility of children to MeHg and the apparent similarities in mechanisms with Hg^0, children are expected to be more susceptible to Hg^0 toxicity as well.

There is a considerable body of evidence from human poisoning episodes that mercury exposure in utero and postnatally results in developmental neurotoxicity (McKeown-Eyssen et al., 1983; Grandjean et al., 1994; Harada, 1995; Grandjean et al., 1997). Thus, infants and children are susceptible subpopulations for adverse health effects from mercury exposure. These effects fall into several general categories: 1) effects on neurological status (Castoldi et al., 2001); 2) age at which developmental milestones are achieved (Marsh et al., 1979); 3) infant and preschool development (Kjellstrom et al., 1986; Kjellstrom et al., 1989); 4) childhood development (age 6 and above) (Grandjean et al., 1997); and 5) sensory or neurophysiological effects (Murata et al., 1999). These studies and others are extensively reviewed by the U.S. EPA (2000) and the NAS (2000)

Whereas MeHg and elemental mercury readily cross the blood-brain barrier and the placental barrier, the mercuric ion (Hg^{2+}) does not readily cross these barriers. However, in fetuses and neonates mercuric species concentrate more in the brain because the blood-brain barrier is incompletely formed. Methylmercury and elemental mercury are lipophilic and are distributed throughout the body. In adults mercuric species accumulate more in the kidney. However, in

neonates mercuric species do not concentrate in the kidneys but are more widely distributed to other tissues (NAS, 2000). It is possible that the increased distribution of mercuric species to the brain in fetuses and neonates accounts for some of the sensitivity of the brain to mercury during these developmental periods. The sensitivity of the fetal brain might also be due to the high proportion of dividing and differentiating cells during neuronal development in the fetal and neonatal periods. These dividing cells may be more sensitive to damaging effects of mercury-protein complexes. Furthermore, neurodevelopment is a "one-way street". Disruption along the route results in permanent deficits. Methylmercury can also alter the relative levels of thyroid hormones to which the fetus is exposed and upon which normal neurodevelopment depends.

In addition to prenatal and postnatal dietary exposure, neonates may receive added postnatal dietary exposure to mercuric species and MeHg from breast milk (Drexler and Schaller, 1998; Sundberg et al., 1999). Animal data suggest that suckling rats retain a higher percentage of ingested organic mercury than do adults, with much higher concentrations in the brain (Kostial et al., 1978). School children can be accidentally exposed to elemental mercury which is a curiosity and an attractive nuisance (George et al., 1996; Lowry et al., 1999). Younger children may also be exposed when elemental mercury is spilled on floors and carpets where they are more active.

6.3 Chronic Toxicity to Experimental Animals

Studies of the effects of mercury in experimental animals generally employ mercury levels in excess of those to which humans are exposed in most settings, thus limiting their ability to model the consequences of long-term, low level exposures. To address this issue, and to test for a role of metallothionein (MT) in mitigating mercury's effects ,Yoshida et al. (2004) exposed wild type and MT-null mice to mercury vapor at 0.06 mg/m^3 (0.007 ppm), 8 hr/day for 23 weeks. Neurobehavioral effects in open field and passive avoidance tests were evaluated at 12 and 23 weeks, and brain levels of mercury were determined. Mercury levels in the brains of mice were 0.66 and 0.97 µg/g tissue for MT-null and wild type, respectively. For comparison, the authors cite human brain mercury levels ranging from 0.3 µg/g in dental personnel to 33 µg/g in retired mercury miners. Mercury-exposed mice showed enhanced motor activity that was statistically significant for both strains at 12 weeks ($p < 0.01$), and for the MT-null mice at 23 weeks ($p < 0.05$). In a learning task (passive avoidance of an electric shock), there were no significant differences between controls and either strain of mouse at 12 weeks of exposure. However, after 23 weeks of exposure, MT-null, but not wild type mice, showed significantly less avoidance than controls ($p < 0.05$) suggesting impaired long-term memory. These data suggest that long-term mercury exposure that results in brain levels of mercury comparable to those seen in occupationally-exposed humans, causes changes in neurobehavior, an effect that is exacerbated by low levels of MT. For comparison, Fawer et al (1983) reported increased intention tremor in human workers exposed to an average of 0.003 ppm for an average of 15 years (Section 6.1).

There is a substantial body of work delineating the neurotoxic effects of MeHg exposure on animals exposed in utero. A comparison between mercury vapor and MeHg, separately and in concert, was conducted in rats. Fredriksson et al. (1996) exposed pregnant rats to MeHg by gavage (2 mg/kg/d during days 6-9 of gestation), and metallic mercury (Hg^0) vapor by inhalation (1.8 mg/m^3 (0.22 ppm) for 1.5 h per day during gestation days 14-19), or both. Controls received the combined vehicles for each of the two treatments. The dose by inhalation was approximately 0.1 mg Hg^0/kg/day. No differences were observed among groups in clinical observations and

developmental markers up to weaning. Tests of behavioral function, performed at 4-5 months of age, included spontaneous motor activity, spatial learning in a circular bath, and instrumental maze learning for food reward. Offspring of dams exposed to Hg^0 showed hyperactivity over all three measures of motor activity: locomotion, rearing and total activity. This effect was enhanced in the animals of the MeHg + Hg^0 group. Compared to either the control or MeHg groups in the swim maze test, rats in the MeHg + Hg^0 and Hg^0 groups took longer to reach a submerged platform whose location they had learned the previous day. Similarly, both the MeHg + Hg^0 and Hg^0 groups showed more ambulations and rearings in the activity test prior to the learning trial in the enclosed radial arm maze. During the learning trial, these same animals showed longer latencies and made more errors in acquiring the food reward. Generally, the results indicated that prenatal exposure to Hg^0 caused alterations to both spontaneous and learned behaviours, suggesting some deficit in adaptive functions. In these experiments, exposure to MeHg was not observed to alter these functions but rather appeared to potentiate the effects of Hg^0.

The similarities in the effects of MeHg and Hg^0 imply similar targets in the brain, which appears to be the case. Pregnant squirrel monkeys were exposed to mercury vapor (0.5 or 1 mg/m^3 (0.06 or 0.12 ppm)) for 4 or 7 hours per day starting in the fifth to the seventh week of gestation and generally ending between 18 and 23 weeks of gestational age (Warfvinge, 2000). The concentration of mercury was found to be higher in maternal (0.80-2.58 µg/g tissue) than in offspring (0.20-0.70 µg/g) brains, but with similar cerebellar distributions. In this study, mercury was localized mainly to Purkinje cells and Bergmann glial cells, similar to the distribution seen after MeHg exposure. The nuclei affected in these and other studies are part of the motor system.

In rats exposed to mercury vapor at ~1 mg/m^3 (0.12 ppm) for 6 h/d, 3 d/wk for 5 wk (low dose), or 24 h/d, 6 d/wk for 5 wk (high dose), an exposure duration-dependent loss of Purkinje cells and proliferation of Bergmann glial cells were observed (Hua et al., 1995). Whereas mercury accumulated to a higher degree in kidney compared to brain, the mercury level in kidney only increased 17% (90 to 105 µg/g tissue) from low to high doses, while that of the brain increased 608% (0.71 to 5.03 µg/g). These neuropathological changes were observed at the same mercury doses as this group reported previously for kidney autoimmune disease (Hua et al., 1993). The brain is a more sensitive target for mercury toxicity in part due to its greater ability to concentrate the metal.

7. Developmental and Reproductive Toxicity

Occupational exposure to mercury vapor has been associated with reproductive problems in a number of epidemiological studies. In a study of 418 dental assistants, Rowland et al. (1994) reported that the fecundability of the women with high exposure to dental amalgams was 63% (95% CI 42-96%) of that reported for the dental assistants with no amalgam exposure. Similarly, in a Chinese study by Yang et al. (2002), there was a significantly higher prevalence of abdominal pain (OR 1.47, 95% CI 1.03: 2.11) and dysmenorrhea (OR 1.66, 95% CI 1.07; 2.59) among female factory workers exposed to ambient mercury vapor (0.001-0.200 mg/m^3) compared with factory workers without mercury exposure. In another study of female factory workers exposed to mercury vapors, the frequency of adverse birth outcomes, especially congenital anomalies, was higher among those exposed to mercury levels at or substantially lower than 0.6 mg/m^3 (Elghany et al., 1997).

The adverse effects of elemental mercury exposure have also been demonstrated in animal models. In rats, elemental mercury readily crosses the placental barrier and accumulates in the fetus following inhalation (Morgan et al., 2002). Pregnant rats exposed by inhalation to 1.8 mg/m³ of metallic mercury for 1 hour or 3 hours/day during gestation (days 11 through 14 plus days 17 through 20) bore pups that displayed significant dose-dependent deficits in behavioral measurements 3-7 months after birth compared to unexposed controls (Danielsson et al., 1993). Behaviors measured included spontaneous motor activity, performance of a spatial learning task, and habituation to the automated test chamber. The pups also showed dose-dependent, increased mercury levels in their brains, livers, and kidneys 2-3 days after birth.

Morgan et al. (2002) exposed pregnant rats for 2 hr per day to 1, 2, 4, or 8 mg/m^3 mercury vapor during gestation days (GD) 6-15, and found a dose-dependent distribution of mercury to all maternal and fetal tissues. Adverse effects on resorptions, postnatal litter size and neonatal body weights were only observed at the highest mercury dose, which was also maternally toxic. It is of interest to note that following cessation of maternal exposure on GD 15, the mass of the fetal brain and its content of mercury both increased 10-fold. Thus the fetal brain continued to accumulate mercury eliminated from maternal tissues. This suggests that the period of fetal exposure is longer than that of maternal exposure, and may affect more neurodevelopmental stages than the timing of the maternal exposure would suggest.

Mercury and mercury compounds, including inorganic forms, are listed under California Proposition 65 (Cal/EPA, Safe Drinking Water and Toxic Enforcement Act of 1986) as developmental toxins. It should be noted that there is substantial evidence in humans of the developmental toxicity of methylmercury exposure. However, this REL summary is meant to be applied to elemental and inorganic mercury, and thus we are not describing methylmercury toxicity in depth in this document.

8. Derivation of Reference Exposure Levels

8.1 Mercury Acute Reference Exposure Level

Study	Danielsson et al., 1993
Study population	groups of 12 pregnant rats
Exposure method	inhalation of metallic mercury vapors
Exposure continuity	
Exposure duration	1 hour per day
Critical effects	CNS disturbances in offspring
LOAEL	1.8 mg/m³
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	
Human Equivalent Concentration	n/a
LOAEL uncertainty factor (UF_L)	10 (default; severe effect, no NOAEL)
Subchronic uncertainty factor (UFs)	
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	√10 (default, animal study)
Toxicodynamic (UF_{A-d})	10 (greater human vs rat susceptibility)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (default: critical study in young)
Toxicodynamic (UF_{H-d})	√10 (default: critical study in young)
Cumulative uncertainty factor	3000
Reference Exposure Level	**0.6 µg Hg/m³ (0.07 ppb Hg^0)**

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document (TSD)).

In the absence of acute inhalation studies in humans, the study by Danielsson et al. (1993) was selected as the critical study since it used a sensitive endpoint, neurotoxicity, in a highly susceptible, developmental stage. Maternal rats were exposed by inhalation to 1.8 mg/m³ of metallic mercury vapor for 1 hour/day or 3 hours/day during gestation. The offspring displayed significant dose-dependent deficits in behavior 3-7 months after birth compared to controls. The default uncertainty factor of 10 is applied for the use of a LOAEL for moderate to severe effects in the absence of a NOAEL.

A default interspecies uncertainty factor of √10 for toxicokinetic (UF_{A-k}) variability was used, while a larger interspecies UF_{A-d} of 10 for toxicodynamic differences was used to reflect the potentially greater developmental susceptibility of humans versus rats. This is based, in part, on Lewandowski et al. (2003) who used a comparative approach to analyze in vivo and in vitro data on the responses of neuronal cells of rats, mice, and humans to MeHg. Their analysis suggests that humans may be up to 10-fold more sensitive to MeHg than are rats. Application of Lewandowski's analysis assumes that the human and rat responses to elemental mercury are comparable with those to MeHg. The study by Fredriksson et al. (1996) (above) supports this

assumption for neurobehavioral effects. A greater susceptibility of humans to adverse neurobehavioral effects following early-life exposures compared with experimental animals has also been seen with other metals, especially lead. For example, Schwartz (1994) reported no evidence for a threshold for neurobehavioral effects in children with blood lead levels of 1 µg/dL compared with less than 15 µg/dL in primates (Gilbert and Rice, 1987) and less than 20 µg/dL in rats (Cory-Slechta et al., 1985).

Since the critical study involved early life exposures, the default intraspecies toxicodynamic uncertainty factor (UF_{H-d}) of $\sqrt{10}$ was employed to account for individual variability. The intraspecies toxicokinetic uncertainty factor of $\sqrt{10}$ reflects the absence of data in young humans, but also the lack of reason to expect major age differences, at least in the short-term kinetics. The resulting acute REL was 0.6 µg/m^3 (0.07 ppb).

This REL is developed for metallic mercury vapor but would be expected to be protective for inhalation of mercury salts. Although mercury salts have no significant vapor pressure under normal atmospheric conditions, they are of concern as hazards if aerosolized or produced during combustion. Animals exposed to mercury vapor inhalation had ten-fold higher brain mercury levels than animals exposed to a similar amount of injected inorganic mercury (mercuric nitrate) (Berlin et al., 1969); however the relationship between kinetics of mercury vapor and mercuric salts has not been extensively studied and may be complex, and dependent on the route, level and timing of exposure.

8.2 Mercury 8-Hour Reference Exposure Level

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 of the Technical Support Document).

The half life of elimination of mercury in humans following a single inhalation exposure of 14-24 min. was 21 days from the head, 64 days from the kidney, and 58 days from the body as a whole (Hursh et al., 1976). Urinary elimination among workers occupationally exposed for several years had an elimination half life of 55 days (Sallsten et al., 1994). Thus, since mercury is only slowly eliminated, the intervals between daily 8-hr exposures, and between weeks are not long enough for the elimination of significant amounts of the metal and it will accumulate in the body with repeated exposure. In view of this bioaccumulative property of mercury exposure in humans, it was considered necessary to use the same study and derivation (in terms of exposure for seven vs only five days per week) for the 8-hour REL as for the chronic REL described below. However, the exposure duration adjustment used in this case reflects a repeated exposure of 8 hours per day with an activity-related air intake of 10 m^3 per day (i.e. half that assumed for a 24-hour period for the chronic REL). As a result, the time-adjusted exposure is twice that for the chronic REL. This adjustment reflects the expectation that activity levels, and hence breathing rates, will be higher during the exposure period than during the remaining 16 hours. The increased breathing rate enhances mercury inhalation during the 8 hour exposure period.

Study	Piikivi and Hanninen (1989); Fawer et al. (1983); Piikivi and Tolonen (1989); Piikivi (1989); Ngim et al. (1992)
Study population	Humans (236)
Exposure method	Inhalation of workplace air
Exposure continuity	8 hours per day, 5 days/week
Exposure duration	13.7 to 15.6 years
Critical effects	Neurotoxicity as measured by: intention tremor; memory and sleep disturbances; decreased performance on neurobehavioral tests (finger tapping, visual scan, visuomotor coordination, visual memory); decreased EEG activity
LOAEL	25 μg/m^3 (3 ppb)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	18 μg/m^3 for LOAEL group (25 x 5/7)
LOAEL uncertainty factor (UF_L)	10 (default, severe effect, no NOAEL)
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (default for inter-individual variability)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children and their developing nervous systems)
Cumulative uncertainty factor	300
Reference Exposure Level	**0.06 μg Hg/m^3 (0.007 ppb Hg^0)**

The studies chosen for determination of the 8-hr REL examined neurotoxicity in humans as a sensitive endpoint following long-term exposures. They all point to a LOAEL of approximately 25 μg/m^3 (3 ppb) with a time-adjusted value of 18 μg/m^3 (25 x 5/7). In the absence of a NOAEL, we applied an uncertainty factor of 10, the default with neurotoxicity considered a moderate to potentially severe effect. The critical study was conducted in humans and was not a subchronic study so no interspecies or subchronic uncertainty factors were applied. To allow for interindividual variability and to specifically account for greater susceptibility among children, an overall intraspecies uncertainty factor of 30 was applied with a toxicokinetic factor (H-k) of √10 to reflect interindividual variability, and a toxicodynamic factor of 10 that reflects the higher susceptibility of the developing nervous system. The cumulative uncertainty is 300, and the resultant 8-hour REL is thus 0.06 μg Hg/ m^3 (0.007 ppb Hg °).

8.3 Mercury Chronic Reference Exposure Level

Study	Piikivi and Hanninen (1989); Fawer et al. (1983); Piikivi and Tolonen (1989); Piikivi (1989); Ngim et al. (1992)
Study population	Humans (236)
Exposure method	Inhalation of workplace air
Exposure continuity	8 hours per day (10 m^3/workday), 5 days/week
Exposure duration	13.7 to 15.6 year
Critical effects	Neurotoxicity as measured by: intention tremor; memory and sleep disturbances; decreased performance on neurobehavioral tests (finger tapping, visual scan, visuomotor coordination, visual memory); decreased EEG activity
LOAEL	25 μg/m^3 (3 ppb)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	9 μg/m^3 for LOAEL group (25 x 10/20 x 5/7)
LOAEL uncertainty factor (UF_L)	10 (default, severe effect, no NOAEL)
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (default for inter-individual variability)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children and their developing nervous systems)
Cumulative uncertainty factor	300
Reference Exposure Level	**0.03 μg Hg/m^3 (0.004 ppb Hg^0)**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

To calculate the chronic REL, studies were chosen that examined a sensitive endpoint (neurotoxicity) in humans following long-term exposures. They all point to a LOAEL of approximately 0.025 mg/m^3 (3 ppb). When adjusted for worker ventilation and workweek exposure, the LOAEL becomes 9 μg/m^3 (25 μg/m^3 x 10 m^3/20 m^3 x 5 d/7 d). In the absence of a NOAEL, we applied an uncertainty factor of 10, the default with neurotoxicity considered a moderate to potentially severe effect. The critical study was conducted in humans and was not a subchronic study so no interspecies or subchronic uncertainty factors were applied. To allow for interindividual variability and to specifically account for greater susceptibility among children, an overall intraspecies uncertainty factor of 30 was applied with a toxicokinetic factor (H-k) of √10 to reflect interindividual variability, and a toxicodynamic factor of 10 that reflects the higher susceptibility of the developing nervous system. The cumulative uncertainty is 300, and the resultant chronic REL is thus 0.03 μg Hg/ m^3 (0.004 ppb Hg °).

The U.S.EPA (1995) based its RfC of 0.3 μg/m^3 (0.04 ppb) on the same study but used an intraspecies uncertainty factor of 3, a LOAEL uncertainty factor of 3 and included a Modifying Factor (MF) of 3 for database deficiencies (lack of developmental and reproductive toxicity data). This modifying factor was not used by OEHHA since allowance was made via the UF_{H-d} for the known sensitivity of children to the neurodevelopmental impacts of mercury.

It is noteworthy that none of the above studies discussed in sufficient detail a dose-response relationship between mercury vapor inhalation and the toxic effects measured. Because none of the studies mention a level below which toxic effects were not seen (a NOAEL), the extrapolation from a LOAEL to a NOAEL should be regarded with caution. Secondly, one study (Ngim et al., 1992) demonstrated neurotoxic effects from mercury inhalation at an exposure level slightly above the other studies, but for a shorter duration. It is possible that mercury could cause neurotoxic effects after a shorter exposure period than that reported in the study used in derivation of the chronic REL.

As mentioned above, OEHHA (1999) has developed a PHG for inorganic mercury in drinking water of 0.0012 mg Hg/L (1.2 ppb) as a level of exposure expected to be without significant health risk from daily water consumption. This value was based on data from a 1993 study by the National Toxicology Program that supported a NOAEL of 0.16 mg Hg/kg-day for renal toxicity in rats with chronic oral exposure. Application of the cumulative uncertainty factor of 1,000 (10 for use of a subchronic study, and 10 each for inter- and intraspecies variability) used in the PHG derivation, gives an oral REL of 0.16 μg Hg/kg-day. This value is several-fold higher than the chronic REL developed above for inhalation of elemental mercury, and reflects the greater ease with which elemental mercury (vs. inorganic mercury) penetrates membranes, especially when exposure is via inhalation versus the oral route.

8.4 Mercury as a Toxic Air Contaminant that Disproportionately Impacts Children

In view of the differential impacts on infants and children identified in Section 6.2.1, and the possibility of direct (inhalation) and indirect exposure (through a diet containing aquatic animals contaminated with methylmercury), OEHHA recommends that elemental mercury be identified as a toxic air contaminant (TAC) which disproportionately impacts children under Health and Safety Code, Section 39699.5.

9. References

ACGIH (1986). Documentation of the Threshold Limit Values and Biological Exposure Indices. Cincinnati (OH): American Conference of Governmental Industrial Hygienists.

Albers JW, Kallenbach LR, Fine LJ, Langolf GD, Wolfe RA, Donofrio PD, Alessi AG, Stolp-Smith KA and Bromberg MB (1988). Neurological abnormalities associated with remote occupational elemental mercury exposure. Ann Neurol 24(5): 651-9.

Allen JW, Mutkus LA and Aschner M (2001). Mercuric chloride, but not methylmercury, inhibits glutamine synthetase activity in primary cultures of cortical astrocytes. Brain Res 891(1-2): 148-57.

Asano S, Eto K, Kurisaki E, Gunji H, Hiraiwa K, Sato M, Sato H, Hasuike M, Hagiwara N and Wakasa H (2000). Acute inorganic mercury vapor inhalation poisoning. Pathol Int 50(3): 169-74.

Ashe W, Largent E, Dutra F, Hubbard D and Blackstone M (1953). Behavior of mercury in the animal organism following inhalation. Ind. Hyg. Occup. Med. 17: 19-43.

Ask K, Akesson A, Berglund M and Vahter M (2002). Inorganic mercury and methylmercury in placentas of Swedish women. Environ Health Perspect 110(5): 523-6.

ATSDR. (1999). Toxicological Profile for Mercury. U.S. Department for Human Health Services. Atlanta, GA

Barregard L, Hultberg B, Schutz A and Sallsten G (1988). Enzymuria in workers exposed to inorganic mercury. Int Arch Occup Environ Health 61(1-2): 65-9.

Berlin M, Fazackerley J and Nordberg G (1969). The uptake of mercury in the brains of mammals exposed to mercury vapor and to mercuric salts. Arch Environ Health 18(5): 719-29.

Bernard AM, Roels HR, Foidart JM and Lauwerys RL (1987). Search for anti-laminin antibodies in the serum of workers exposed to cadmium, mercury vapour or lead. Int Arch Occup Environ Health 59(3): 303-9.

Campbell J (1948). Acute mercurial poisoning by inhalation of metallic vapour in an infant. Can Med Assoc J 58: 72-75.

CARB (2005a). Annual Statewide Toxics Summary - mercury http://www.arb.ca.gov/aqd/toxics/statepages/hgstate.html. Sacramento, CA.

CARB. (2005b). *California Toxics Inventory for 2004*. California Air Resources Board. http://www.arb.ca.gov/toxics/cti/cti.htm.

Castoldi AF, Coccini T, Ceccatelli S and Manzo L (2001). Neurotoxicity and molecular effects of methylmercury. Brain Res Bull 55(2): 197-203.

Choi BH (1989). The effects of methylmercury on the developing brain. Prog Neurobiol 32(6): 447-70.

Cordier S, Grasmick C, Paquier-Passelaigue M, Mandereau L, Weber JP and Jouan M (1998). Mercury exposure in French Guiana: levels and determinants. Arch Environ Health 53(4): 299-303.

Cory-Slechta DA, Weiss B and Cox C (1985). Performance and exposure indices of rats exposed to low concentrations of lead. Toxicol Appl Pharmacol 78(2): 291-9.

Counter SA, Buchanan LH, Laurell G and Ortega F (1998). Blood mercury and auditory neuro-sensory responses in children and adults in the Nambija gold mining area of Ecuador. Neurotoxicology 19(2): 185-96.

Danielsson BR, Fredriksson A, Dahlgren L, Gardlund AT, Olsson L, Dencker L and Archer T (1993). Behavioural effects of prenatal metallic mercury inhalation exposure in rats. Neurotoxicol Teratol 15(6): 391-6.

Drexler H and Schaller KH (1998). The mercury concentration in breast milk resulting from amalgam fillings and dietary habits. Environ Res 77(2): 124-9.

Elghany NA, Stopford W, Bunn WB and Fleming LE (1997). Occupational exposure to inorganic mercury vapour and reproductive outcomes. Occup Med (Lond) 47(6): 333-6.

Fagala GE and Wigg CL (1992). Psychiatric manifestations of mercury poisoning. J Am Acad Child Adolesc Psychiatry 31(2): 306-11.

Fawer RF, de Ribaupierre Y, Guillemin MP, Berode M and Lob M (1983). Measurement of hand tremor induced by industrial exposure to metallic mercury. Br J Ind Med 40(2): 204-8.

Florentine MJ and Sanfilippo DJ, 2nd (1991). Elemental mercury poisoning. Clin Pharm 10(3): 213-21.

Fredriksson A, Dencker L, Archer T and Danielsson BR (1996). Prenatal coexposure to metallic mercury vapour and methylmercury produce interactive behavioural changes in adult rats. Neurotoxicol Teratol 18(2): 129-34.

George L, Scott FE, Cole D, Siracusa L, Buffett C, Hunter W and Zinkewich R (1996). The mercury emergency and Hamilton school children: a follow-up analysis. Can J Public Health 87(4): 224-6.

Gilbert SG and Rice DC (1987). Low-level lifetime lead exposure produces behavioral toxicity (spatial discrimination reversal) in adult monkeys. Toxicol Appl Pharmacol 91(3): 484-90.

Grandjean P, Weihe P and Nielsen JB (1994). Methylmercury: significance of intrauterine and postnatal exposures. Clin Chem 40(7 Pt 2): 1395-400.

Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sorensen N, Dahl R and Jorgensen PJ (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 19(6): 417-28.

Grandjean P, White RF, Nielsen A, Cleary D and de Oliveira Santos EC (1999). Methylmercury neurotoxicity in Amazonian children downstream from gold mining. Environ Health Perspect 107(7): 587-91.

Hacon S, Artaxo P, Gerab F, Yamasoe MA, Campos RC, Conti LF and De Lacerda LD (1995). Atmospheris mercury and trace elements in the region of Alta Floresta in the Amazon basin. Water, Air, and Soil Pollution 80(1-4): 273-283.

Harada M (1995). Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit Rev Toxicol 25(1): 1-24.

Hua J, Brun A and Berlin M (1995). Pathological changes in the Brown Norway rat cerebellum after mercury vapour exposure. Toxicology 104(1-3): 83-90.

Hua J, Pelletier L, Berlin M and Druet P (1993). Autoimmune glomerulonephritis induced by mercury vapour exposure in the Brown Norway rat. Toxicology 79(2): 119-29.

Hursh JB, Cherian MG, Clarkson TW, Vostal JJ and Mallie RV (1976). Clearance of mercury (HG-197, HG-203) vapor inhaled by human subjects. Arch Environ Health 31(6): 302-9.

Kanluen S and Gottlieb CA (1991). A clinical pathologic study of four adult cases of acute mercury inhalation toxicity. Arch Pathol Lab Med 115(1): 56-60.

Kishi R, Doi R, Fukuchi Y, Satoh H, Satoh T, Ono A, Moriwaka F, Tashiro K and Takahata N (1993). Subjective symptoms and neurobehavioral performances of ex-mercury miners at an average of 18 years after the cessation of chronic exposure to mercury vapor. Mercury Workers Study Group. Environ Res 62(2): 289-302.

Kjellstrom T, Kennedy P, Wallis S, Stewart A, Friberg L, Lind B, Wutherspoon T and Mantell C. (1989). Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2: Interviews and psychological tests at age 6. Report 3642. National Swedish Environmental Protection Board. Solna, Sweden

Kjellstrom T, Kennedy S, Wallis S and Mantell C. (1986). Physical and mental development of children with prenatal exposure to mercury from fish. Stage I: Preliminary tests at age 4. Report #3080. National Swedish Environmental Protection Board. Solna, Sweden

Kostial K, Kello D, Jugo S, Rabar I and Maljkovic T (1978). Influence of age on metal metabolism and toxicity. Environ Health Perspect 25: 81-6.

Langolf GD, Chaffin DB, Henderson R and Whittle HP (1978). Evaluation of workers exposed to elemental mercury using quantitative tests of tremor and neuromuscular functions. Am Ind Hyg Assoc J 39(12): 976-84.

Leggett RW, Munro NB and Eckerman KF (2001). Proposed revision of the ICRP model for inhaled mercury vapor. Health Phys 81(4): 450-5.

Lerch M and Bircher AJ (2004). Systemically induced allergic exanthem from mercury. Contact Dermatitis 50(6): 349-53.

Levin M, Jacobs J and Polos PG (1988). Acute mercury poisoning and mercurial pneumonitis from gold ore purification. Chest 94(3): 554-6.

Lewandowski TA, Ponce RA, Charleston JS, Hong S and Faustman EM (2003). Effect of methylmercury on midbrain cell proliferation during organogenesis: potential cross-species differences and implications for risk assessment. Toxicol Sci 75(1): 124-33.

Liang YX, Sun RK, Sun Y, Chen ZQ and Li LH (1993). Psychological effects of low exposure to mercury vapor: application of a computer-administered neurobehavioral evaluation system. Environ Res 60(2): 320-7.

Livardjani F, Ledig M, Kopp P, Dahlet M, Leroy M and Jaeger A (1991). Lung and blood superoxide dismutase activity in mercury vapor exposed rats: effect of N-acetylcysteine treatment. Toxicology 66(3): 289-95.

Lowry LK, Rountree PP, Levin JL, Collins S and Anger WK (1999). The Texarkana mercury incident. Tex Med 95(10): 65-70.

Lund BO, Miller DM and Woods JS (1993). Studies on Hg(II)-induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem Pharmacol 45(10): 2017-24.

Marsh DO, Myers GJ, Clarkson TW, Amin-Zaki L, Tikriti S and Majeed MA (1979). Fetal methylmercury poisoning: clinical and toxicological data on 29 cases. Ann Neurol 7(4): 348-53.

Matthes FT, Kirschner R, Yow MD and Brennan JC (1958). Acute poisoning associated with inhalation of mercury vapor; report of four cases. Pediatrics 22(4 Part 1): 675-88.

McFarland RB and Reigel H (1978). Chronic mercury poisoning from a single brief exposure. J Occup Med 20(8): 532-4.

McKeown-Eyssen GE, Ruedy J and Neims A (1983). Methyl mercury exposure in northern Quebec. II. Neurologic findings in children. Am J Epidemiol 118(4): 470-9.

Morgan DL, Chanda SM, Price HC, Fernando R, Liu J, Brambila E, O'Connor RW, Beliles RP and Barone S, Jr. (2002). Disposition of inhaled mercury vapor in pregnant rats: maternal toxicity and effects on developmental outcome. Toxicol Sci 66(2): 261-73.

Murata K, Weihe P, Renzoni A, Debes F, Vasconcelos R, Zino F, Araki S, Jorgensen PJ, White RF and Grandjean P (1999). Delayed evoked potentials in children exposed to methylmercury from seafood. Neurotoxicol Teratol 21(4): 343-8.

Nakayama H, Niki F, Shono M and Hada S (1983). Mercury exanthem. Contact Dermatitis 9(5): 411-7.

NAS. (2000). Toxicological Effects of Methyl Mercury. National Academy of Sciences. Washington D.C.

Netterstrom B, Guldager B and Heeboll J (1996). Acute mercury intoxication examined with coordination ability and tremor. Neurotoxicol Teratol 18(4): 505-9.

Ngim CH, Foo SC, Boey KW and Jeyaratnam J (1992). Chronic neurobehavioural effects of elemental mercury in dentists. Br J Ind Med 49(11): 782-90.

OEHHA. (1999). Public Health Goal for Inorganic Mercury in Drinking Water. Office of Environmental Health Hazard Assessment. California Environmental Protection Agency. Scramento, CA

Pamphlett R and Coote P (1998). Entry of low doses of mercury vapor into the nervous system. Neurotoxicology 19(1): 39-47.

Piikivi L (1989). Cardiovascular reflexes and low long-term exposure to mercury vapour. Int Arch Occup Environ Health 61(6): 391-5.

Piikivi L and Hanninen H (1989). Subjective symptoms and psychological performance of chlorine-alkali workers. Scand J Work Environ Health 15(1): 69-74.

Piikivi L, Hanninen H, Martelin T and Mantere P (1984). Psychological performance and long-term exposure to mercury vapors. Scand J Work Environ Health 10(1): 35-41.

Piikivi L and Ruokonen A (1989). Renal function and long-term low mercury vapor exposure. Arch Environ Health 44(3): 146-9.

Piikivi L and Tolonen U (1989). EEG findings in chlor-alkali workers subjected to low long term exposure to mercury vapour. Br J Ind Med 46(6): 370-5.

Rodgers JS, Hocker JR, Hanas RJ, Nwosu EC and Hanas JS (2001). Mercuric ion inhibition of eukaryotic transcription factor binding to DNA. Biochem Pharmacol 61(12): 1543-50.

Roels H, Lauwerys R, Buchet JP, Bernard A, Barthels A, Oversteyns M and Gaussin J (1982). Comparison of renal function and psychomotor performance in workers exposed to elemental mercury. Int Arch Occup Environ Health 50(1): 77-93.

Rowland AS, Baird DD, Weinberg CR, Shore DL, Shy CM and Wilcox AJ (1994). The effect of occupational exposure to mercury vapour on the fertility of female dental assistants. Occup Environ Med 51(1): 28-34.

Rowley B and Monestier M (2005). Mechanisms of heavy metal-induced autoimmunity. Mol Immunol 42(7): 833-8.

Sallsten G, Barregard L and Schutz A (1994). Clearance half life of mercury in urine after the cessation of long term occupational exposure: influence of a chelating agent (DMPS) on excretion of mercury in urine. Occup Environ Med 51(5): 337-42.

Schwartz J (1994). Low-level lead exposure and children's IQ: a meta-analysis and search for a threshold. Environ Res 65(1): 42-55.

Sexton DJ, Powell KE, Liddle J, Smrek A, Smith JC and Clarkson TW (1978). A nonoccupational outbreak of inorganic mercury vapor poisoning. Arch Environ Health 33(4): 186-91.

Smith RG, Vorwald AJ, Patil LS and Mooney TF, Jr. (1970). Effects of exposure to mercury in the manufacture of chlorine. Am Ind Hyg Assoc J 31(6): 687-700.

Sundberg J, Jonsson S, Karlsson MO and Oskarsson A (1999). Lactational exposure and neonatal kinetics of methylmercury and inorganic mercury in mice. Toxicol Appl Pharmacol 154(2): 160-9.

Taueg C, Sanfilippo DJ, Rowens B, Szejda J and Hesse JL (1992). Acute and chronic poisoning from residential exposures to elemental mercury--Michigan, 1989-1990. J Toxicol Clin Toxicol 30(1): 63-7.

Teisinger J and Fiserova-Bergerova V (1965). Pulmonary retention and excretion of mercury vapors in man. Ind Med Surg 34: 580-4.

Tjalve H and Henriksson J (1999). Uptake of metals in the brain via olfactory pathways. Neurotoxicology 20(2-3): 181-95.

Torres AD, Rai AN and Hardiek ML (2000). Mercury intoxication and arterial hypertension: report of two patients and review of the literature. Pediatrics 105(3): E34.

U.S.EPA. (2000). Reference Dose for Mercury. External Review Draft. NCEA-S-0930. National Center for Environmental Assessment.

USEPA. (1995). *Mercury, elemental Reference concentration for chronic inhalation exposure (RfC)* http://www.epa.gov/iris/subst/0370.htm.

USEPA. (1997). Mercury Study Report to Congress. Health Effects of Mercury and Mercury Compounds (Vol V.). Office of Air Quality Planning and Standards; Office of Research and Development.

Warfvinge K (2000). Mercury distribution in the neonatal and adult cerebellum after mercury vapor exposure of pregnant squirrel monkeys. Environ Res 83(2): 93-101.

Warfvinge K, Hansson H and Hultman P (1995). Systemic autoimmunity due to mercury vapor exposure in genetically susceptible mice: dose-response studies. Toxicol Appl Pharmacol 132(2): 299-309.

Yang JM, Chen QY and Jiang XZ (2002). Effects of metallic mercury on the perimenstrual symptoms and menstrual outcomes of exposed workers. Am J Ind Med 42(5): 403-9.

Yole M, Wickstrom M and Blakley B (2007). Cell death and cytotoxic effects in YAC-1 lymphoma cells following exposure to various forms of mercury. Toxicology 231(1): 40-57.

Yoshida M, Satoh M, Shimada A, Yasutake A, Sumi Y and Tohyama C (1999). Pulmonary toxicity caused by acute exposure to mercury vapor is enhanced in metallothionein-null mice. Life Sci 64(20): 1861-7.

Yoshida M, Watanabe C, Satoh M, Yasutake A, Sawada M, Ohtsuka Y, Akama Y and Tohyama C (2004). Susceptibility of metallothionein-null mice to the behavioral alterations caused by exposure to mercury vapor at human-relevant concentration. Toxicol Sci 80(1): 69-73.